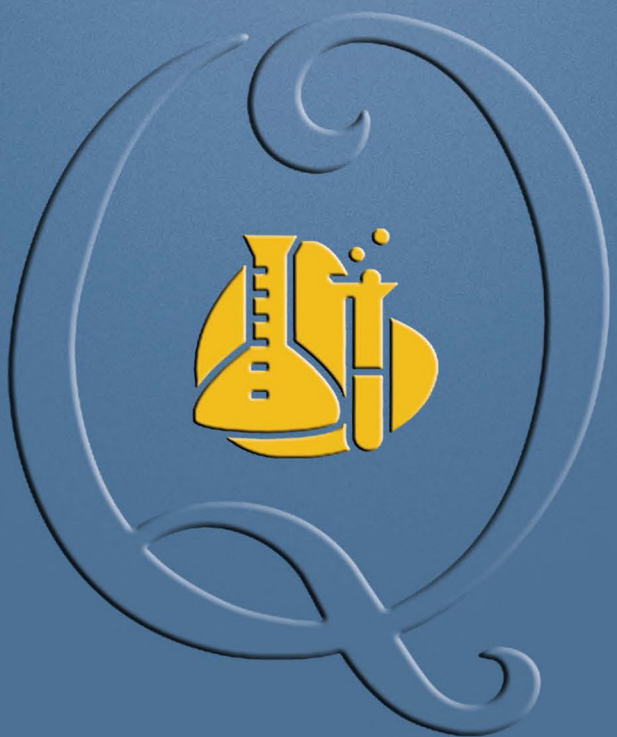
ANALYTICAL CHEMISTRY

CRC Press
Taylor & Francis Group

Quality Assurance and Quality Control in the Analytical Chemical Laboratory

A Practical Approach

THIRD EDITION



PIOTR KONIECZKA

Quality Assurance and Quality Control in the Analytical Chemical Laboratory

The third edition of *Quality Assurance and Quality Control in the Analytical Chemical Laboratory: A Practical Approach* defines the tools used in quality assurance/quality control (QA/QC), especially the application of statistical tools during analytical data treatment. Clearly written and logically organized, this well-loved volume takes a generic approach applicable to any field of analysis. The author begin with the theory behind quality control systems, then detail validation parameter measurements, the use of statistical tests, counting the margin of error, uncertainty estimation, traceability, reference materials, proficiency tests and method validation. The new edition contains fully updated references throughout and includes new information on certified reference material (CRMs) and proficiency tests (PTs). A new chapter covers calibration and contains numerous new examples, and the subject of accreditation is expanded.

- Fully updated and revised references.
- New computational examples and solution problems.
- New chapter on calibration and expanded coverage of accreditation.
- A practical approach applicable to any field of analysis.

Analytical Chemistry

Quality Assurance and Quality Control in the Analytical Chemical Laboratory

Piotr Konieczka

Quality Assurance and Quality Control in the Analytical Chemical Laboratory

A Practical Approach

Third Edition

Piotr Konieczka

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

Third edition published 2026
by CRC Press
2385 NW Executive Center Drive, Suite 320, Boca Raton FL 33431

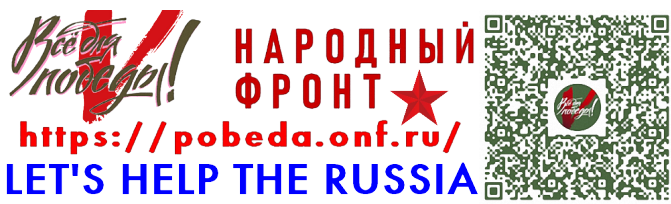
and by CRC Press
4 Park Square, Milton Park, Abingdon, Oxon, OX14 4RN

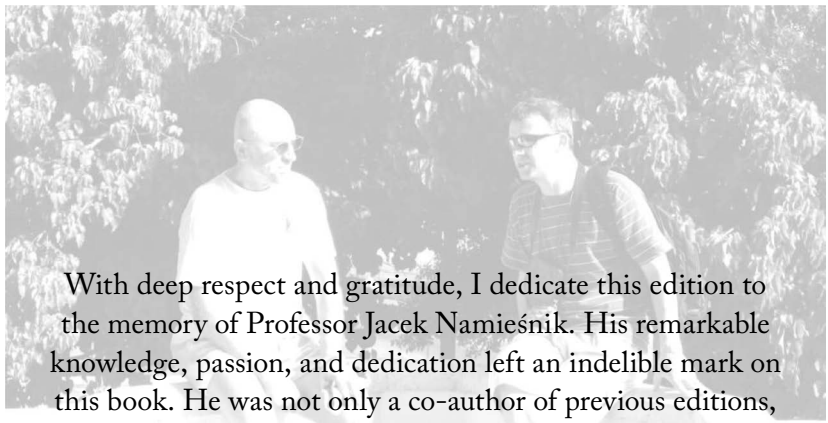
CRC Press is an imprint of Taylor & Francis Group, LLC

ISBN: 978-1-032-82465-9 (hbk)
ISBN: 978-1-032-82466-6 (pbk)
ISBN: 978-1-003-50462-7 (ebk)

DOI 10.1201/9781003504627

Typeset in Caslon
by KnowledgeWorks Global Ltd.





With deep respect and gratitude, I dedicate this edition to the memory of Professor Jacek Namieśnik. His remarkable knowledge, passion, and dedication left an indelible mark on this book. He was not only a co-author of previous editions, but also an inspiration, a mentor, and a dear friend. Though he is no longer with us, his contributions live on in these pages. I am forever grateful for his insights, ideas, and the invaluable role he played in shaping this work. May this publication stand as a tribute to his legacy and the impact he had on all of us.

Piotr Konieczka

Contents

About the Author

Prof. Piotr Konieczka, PhD, DSc Head of the Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology

Research interests include environmental pollution analysis, trace analysis, in particular analysis of heavy metal content using spectroscopic techniques, as well as chemical statistics and metrology, as well as control and quality assurance of analytical measurement results, including aspects related to the production and use of certified reference materials (CRM) and the organization and development of proficiency test results (PT).

Supervisor/co-supervisor in completed nine PhDs and three more open.

Author, co-author of 116 publications from the Philadelphia list, eight books, one patent,

total Hirsch index = 26 (WoS 18.02.2025), number of citations (without self-citations) 4208 (WoS 18.02.2025)

total Hirsch index = 27 (Scopus 18.02.2025), number of citations (without self-citations) 5121 (Scopus 18.02.2025).

Preface

The aim of this book is to provide practical information about quality assurance/quality control (QA/QC) systems, including definition of all tools, understanding of their uses and an increase in knowledge about the practical application of statistical tools during analytical data treatment.

Although this book is primarily designed for students and academic teachers, it may also prove useful to the scientific community, particularly among those who are interested in QA/QC. With its comprehensive coverage, this book can be of particular interest to researchers in the industry and academia, as well as government agencies and legislative bodies.

The theoretical part of the book contains information on questions relating to quality control systems.

The practical part includes more than 90 examples relating to validation parameter measurements, using statistical tests, calculation of the margin of error, estimating uncertainty, etc. For all examples, a constructed calculation datasheet (Excel) is attached, which makes problem-solving easier.

The eResource files available to readers of this text contain more than 80 Excel datasheet files, each consisting of three main components: problem, data and solution; in some cases, additional data such as graphs and conclusion are also included. After saving an Excel file

on the hard disk, it is possible to use it on different data sets. It should be noted that in order to obtain correct calculations, it is necessary to use it appropriately. The user's own data should be copied only into yellow marked cells (be sure that your data set fits the appropriate datasheet). Solution data will be calculated and can be read from green marked cells.

I hope that with this book, I can contribute to a better understanding of all problems connected with QA/QC.

Abbreviations

AAS	Atomic Absorption Spectrometry
ANOVA	ANalysis Of VAriance
BCR	Bureau Communautaire de Reference (standards, measurements and testing programme–European community)
CDF	Cumulative Distribution Function
CITAC	Cooperation on International Traceability in Analytical Chemistry
CL	Central Line
CRM	Certified Reference Material
CUSUM	CUmulative SUM
CV	Coefficient of Variation
CVAAS	Cold Vapor Atomic Absorption Spectrometry
D	mean absolute Deviation
EN	European Norm
GC	Gas Chromatography
GLP	Good Laboratory Practice
GUM	Guide to the expression of Uncertainty in Measurement
IAEA	International Atomic Energy Agency
ICH	International Conference on Harmonisation
IDL	Instrumental Detection Limit
ILC	Interlaboratory Comparisons

IQC	Internal Quality Control
IQR	InterQuaRtile value
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JCGM	Joint Committee for Guides in Metrology
LAL	Lower Action (control) Limit
LOD	Limit of Detection
LOQ	Limit of Quantification
L-PS	Laboratory-Performance Study
LRM	Laboratory Reference Material
LWL	Lower Warning Limit
MB	Method Blank
M-CS	Material-Certification Study
MDL	Method Detection Limit
Me	Median
Mo	Mode
M-PS	Method-Performance Study
MQL	Method Quantification Limit
NIES	National Institute for Environmental Studies
NIST	National Institute of Standards and Technology
NRCC	National Research Council of Canada
PRM	Primary Reference Material
PT	Proficiency Test
q	quartile
$QA_{(1)}$	Quality Assessment
$QA_{(2)}$	Quality Assurance
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
QCM	Quality Control Material
r	regression coefficient (correlation coefficient)
R	range
RH	Relative Humidity
RM	Reference Material
RSD	Relative Standard Deviation
S/N	Signal to Noise ratio
SD	Standard Deviation
SecRM	Secondary Reference Material

SI	Le Systeme Internationale d'Unités (The International System of Units)
SOP	Standard Operating Procedure
SRM	Standard Reference Material
UAL	Upper Action (control) Limit
USP	The United States Pharmacopea
UWL	Upper Warning Limit
V	Variance
VIM	Vocabulaire International des Termes Fondamentaux et Généraux de Métrologie (International Vocabulary of Metrology)
VIRM	The European Virtual Institute for Reference Materials
%R	Recovery

1
Basic Notions of Statistics

1.1 Introduction

Mathematical statistics is a branch of mathematics that applies the theory of probability to examining regularities in the occurrence of certain properties of material objects or phenomena which occur in unlimited quantities. Statistics presents these regularities by means of numbers.

Statistics is not only art for art's sake. It is a very useful tool that can help us find answers to many questions. Statistics is especially helpful for analysts because it may clear many doubts and answer many questions associated with the nature of an analytic process, for example:

- how exact the result of determination is,
- how many determinations should be conducted to increase the precision of a measurement,
- whether the investigated product fulfills the necessary requirements and/or norms.

Yet, it is important to remember that statistics should be applied in a reasonable way.

1.2 Distributions of Random Variables

1.2.1 Characterization of Distributions

The application of a certain analytical method unequivocally determines the distribution of measurement results (properties), here treated as independent random variables. A result is a consequence of a measurement. The set of obtained determination results creates a distribution (empirical).

Each defined distribution is characterized by the following parameters:

- a cumulative distribution function (*CDF*) X is determined by F_X and represents the probability that a random variable X takes on a value less than or equal to x; a *CDF* is (not necessarily strictly) right-continuous, with its limit equal to 1 for arguments approaching positive infinity, and equal to 0 for arguments approaching negative infinity; in practice, a *CDF* is described shortly by: $F_X(x) = P(X \leq x)$,
- a density function which is the derivative of the *CDF*: $f(x) = F'_X(x)$.

Below are the short characterizations of the most frequently used distributions:

- normal distribution,
- uniform distribution (rectangular),
- triangular distribution.

Normal distribution, also called the Gaussian distribution (particularly in physics and engineering), is a very important probability distribution used in many domains. It is an infinite family of many distributions, defined by two parameters: mean (location) and standard deviation (scale).

Normal distribution, $N(\mu_x, SD)$ is characterized by the following properties:

- an expected value μ_x,
- a median $Me = \mu_x$,
- a variance V.

Uniform distribution (also called continuous or rectangular) is a continuous probability distribution for which the probability density function within the interval $\langle -a, +a \rangle$ is constant and not equal to zero, but outside, the interval is equal to zero.

Because this distribution is continuous, it is not important whether the endpoints $-a$ and $+a$ are included in the interval. The distribution is determined by a pair of parameters $-a$ and $+a$.

Uniform distribution is characterized by:

- an expected value $\mu_x = 0$,
- a median $Me = 0$,
- a variance $V = a^2/3$.

Triangular distribution over the interval $\langle -a, +a \rangle$ is characterized by:

- an expected value $\mu_x = 0$,
- a median $Me = 0$,
- a variance $V = a^2/6$.

The distribution of a random variable provides complete information on an investigated characteristic (e.g. concentration, content, physiochemical property). Unfortunately, such complete information is seldom available. As a rule, characteristic inference is drawn using the analysis of a limited number of elements (samples) representing a fragment of the whole set that is described by the distribution. Then, one may infer a characteristic using an estimation of some of its parameters (statistical parameters) or its empirical distribution.

Statistical parameters are numerical quantities used in the systematic description of a statistical population structure.

These parameters can be divided into four basic groups:

- measures of location,
- measures of statistical dispersion,
- measures of asymmetry,
- measures of concentration.

1.3 Measures of Location

Measures of location use one value to characterize the general level of the value of the characteristic in a population [1].

The most popular measures of location are the following:

- arithmetic mean,
- truncated mean,
- mode,
- quantiles:
  - quartiles,
  - median,
  - deciles.

Arithmetic mean is the sum of all the values of a measurable characteristic divided by the number of units in a finite population:

$$x_m = \frac{\sum_{i=1}^{n} x_i}{n} \tag{1.1}$$

Here are the selected properties of the arithmetic mean:

- the sum of the values is equal to the product of the arithmetic mean and the population size,
- the arithmetic mean fulfills the condition:

$$x_{min} < x_m < x_{max} \tag{1.2}$$

- the sum of deviations of individual values from the mean is equal to zero:

$$\sum_{i=1}^{n} \left(x_i - x_m \right) = 0 \tag{1.3}$$

- the sum of squares of deviations of each value from the mean is minimal:

$$\sum_{i=1}^{n} \left(x_i - x_m \right)^2 = min \tag{1.4}$$

- the arithmetic mean is sensitive to extreme values (outliers) of the characteristic,
- the arithmetic mean from a sample is a good approximation (estimation, estimator) of the expected value.

The **truncated mean** x_{wk} is a statistical measurement calculated for the series of results, among which the extrema (minima or maxima) have a high uncertainty concerning their actual value [2]. Its value is calculated according to the formula:

$$x_{wk} = \frac{1}{n} \left[\left(k+1 \right) x_{(k+1)} + \sum_{i=k+2}^{n-k-1} x_{(i)} + \left(k+1 \right) x_{(n-k)} \right] \tag{1.5}$$

where:

x_{wk} – truncated mean,
n – number of results in the series,
k – number of extreme (discarded) results.

Mode *Mo* is the value that occurs most frequently in a data set. In a set of results, there may be more than one value that can be a mode because the same maximum frequency can be attained at different values.

Quantiles *q* are values in an investigated population (a population presented in the form of a statistical series) that divide the population into a certain number of subsets. Quantiles are data values marking boundaries between consecutive subsets.

A quantile of order one half is otherwise known as a **median**, quantiles of order one fourths, two fourths, three fourths are otherwise known as **quartiles**, quantiles of the order one tenth, two tenth, …, nine tenth are otherwise known as **deciles** and quantiles of the order one hundredth, two hundredth, …, ninety nine hundredth are otherwise known as **percentiles**.

A **quartile** is any of three values which divide a sorted data set into four equal parts, so that each part represents one-fourth of the sampled population.

The first quartile (designated q_1) divides the population in a such a way that 25% of the population units have values lower than or equal to the first quartile q_1, and 75% units have values higher than or equal to the first quartile. The second quartile q_2 is the median. The third quartile (designated q_3) divides the population in a such a way that 75% of the population units have values lower than or equal to the third quartile q_3, and 25% units have values higher than or equal to the quartile.

The **median *Me*** measurement is the middle number in a population arranged in a non-decreasing order (for a population with an odd number of observations), or the mean of the two middle values (for those with an even number of observations).

A median separates the higher half of a population from the lower half; half of the units have values smaller than or equal to the median, and half of them have values higher than or equal to the median. Contrary to the arithmetic mean, the median is not sensitive to outliers in a population. This is usually perceived as its advantage but sometimes may also be regarded as a flaw; even immense differences between outliers and the arithmetic mean do not affect its value.

Hence, other means have been proposed, for example, the truncated mean. This mean, less sensitive to outliers than the standard mean (only a large number of outliers can significantly influence the truncated mean) and standard deviation, is calculated using all results,

which transfers the extreme to an accepted deviation range – thanks to the application of appropriate iterative procedures.

The first **decile** represents 10% of the results which have values lower than or equal to the first decile, and 90% of the results have values greater than or equal to it.

1.4 Measures of Dispersion

Measures of dispersion (variability) are usually used to determine differences between individual observations and mean value [1].

The most popular measures of dispersion are as follows:

- range,
- variance,
- standard deviation,
- average deviation,
- coefficient of variation,

The **range *R*** is a difference between the maximum and minimum value of an examined characteristic:

$$R = x_{max} - x_{min} \tag{1.6}$$

It is a measure characterizing the empirical variability region of the examined characteristic but does not give information on the variability of individual values of the characteristic in the population.

Variance *V* is an arithmetic mean of the squared distance of values from the arithmetic mean of the population. Its value is calculated according to the formula:

$$V = \frac{1}{n-1}\sum_{i-1}^{n}\left(x_i - x_m\right)^2 \tag{1.7}$$

Standard deviation *SD*, the square root of the variance, is the measure of dispersion of individual results around the mean. It is described by the equation:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - x_m\right)^2}{n-1}} \tag{1.8}$$

Standard deviation equals zero only when all results are identical. In all other cases, it has positive values. Thus, the greater the dispersion of results, the greater the value of the *SD*.

It must be remembered that dispersion of results occurs in each analytical process. Yet, it is not always observed, for example, because of the resolution of a measuring instrument being too low.

Properties of the standard deviation are

- if a constant value is added to or subtracted from each value, the standard deviation does not change,
- if each measurement value is multiplied or divided by any constant value, the standard deviation is also multiplied/divided by that same constant,
- standard deviation is always a denominate number, and it is always expressed in the same units as the results.

If an expected value μ_x is known, the standard deviation is calculated according to the following formula:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - \mu_x\right)^2}{n}} \tag{1.9}$$

Relative standard deviation (*RSD*) is obtained by dividing the standard deviation by the arithmetic mean:

$$RSD = \frac{SD}{x_m} \tag{1.10}$$

obviously $x_m \neq 0$.

The standard deviation of the arithmetic mean $\overline{SD}$ is calculated according to the following equation:

$$\overline{SD} = \frac{SD}{\sqrt{n}} \tag{1.11}$$

The standard deviation of an analytical method SD_g (general) is determined using the results from a series of measurements:

$$SD_g = \sqrt{\frac{1}{n-k}\sum_{i=1}^{k} SD_i^2\left(n_i - 1\right)} \tag{1.12}$$

where:

k – the number of series of parallel determinations.

For series with equal numbers of elements, the formula is simplified to the following equation:

$$SD_g = \sqrt{\frac{1}{k}\sum_{i=1}^{k} SD_i^2} \tag{1.13}$$

The **mean absolute deviation *D*** is an arithmetic mean of absolute deviations of the values from the arithmetic mean. It determines the mean difference between the results in the population and the arithmetic mean:

$$D = \frac{1}{n}\sum_{i=1}^{n}\left|x_i - x_m\right| \tag{1.14}$$

The relationship between the mean and standard deviations for the same set of results can be presented as $D < SD$.

The **coefficient of variation *CV*** is *RSD* presented in %:

$$CV = RSD\ [\%] \tag{1.15}$$

The CV is the quotient of the absolute variation measure of the investigated characteristic and the mean value of that characteristic. It is an absolute number, usually presented in percentage points.

The CV is usually applied in comparing differences:

- between several populations with regard to the same characteristic,
- within the same population with regard to a few different characteristics.

1.5 Measures of Asymmetry

A **skewness (asymmetry) coefficient** is an absolute value expressed as the difference between an arithmetic mean and a mode.

The skewness coefficients are applied in comparisons in order to estimate the force and the direction of asymmetry. These are absolute numbers: the greater asymmetry, the greater their value.

The quartile skewness coefficient shows the direction and force of result asymmetry located between the first and third quartiles.

1.6 Measures of Concentration

A **concentration coefficient *K*** is a measure of the concentration of individual observations around the mean. The greater the value of the coefficient, the more slender the frequency curve and the greater the concentration of the values about the mean.

Example 1.1

Problem: For the given series of measurement results, give the following values:

- mean
- standard deviation
- relative standard deviation
- mean absolute deviation
- coefficient of variation
- minimum
- maximum
- range
- median
- mode

Data: result series, mg/dm^3:

1	12.34	**8**	12.34
2	12.67	**9**	12.00
3	12.91	**10**	12.67
4	12.02	**11**	12.53
5	12.52	**12**	12.34
6	12.12	**13**	12.79
7	12.98		

SOLUTION:

Mean, x_m, mg/dm^3	12.48
Standard deviation, *SD*, mg/dm^3	0.32
Relative standard deviation, *RSD*	0.026
Mean absolute deviation, *D*	0.26
Coefficient of variation – *CV*, %	2.6%
Minimum, x_{min}, mg/dm^3	12.00
Maximum, x_{max}, mg/dm^3	12.98
Range, *R*, mg/dm^3	0.98
Median, *Me*, mg/dm^3	12.52
Mode, *Mo*, mg/dm^3	12.34

Excel file: exampl_1_1.xls

1.7 Statistical Hypothesis Testing

A hypothesis is a proposition concerning a population, based on probability, assumed in order to explain some phenomenon, law or fact. A hypothesis requires testing.

Statistical hypothesis testing means checking propositions with regard to a population which have been formulated without examining the whole population. The plot of the testing procedure involves:

1. Formulating the null hypothesis and the alternative hypothesis
   The null hypothesis H_o is a simple form of the hypothesis that is subjected to tests,
   The alternative hypothesis H_1 is contrasted with the null hypothesis.
2. The choice of an appropriate test.
   The test serves to verify the hypothesis.
3. Determination of the level of significance α
4. Determining the critical region of a test
   The size of the critical region is determined by any low level of significance α, and its location is determined by the alternative hypothesis.
5. Calculation of a test's parameter using a sample
   The results of the sample are processed in an appropriate manner, according to the procedure of the selected test and are the basis for the calculation of the test statistic.

6. Conclusion

   The test statistic, determined using the sample, is compared with the critical value of the test:

   - if the value falls within the critical region, then the null hypothesis should be rejected as false. It means that the value of the calculated test parameter is greater than the critical value of the test (read from a relevant table),
   - if the value is outside the critical region, it means that there is not enough evidence to reject the null hypothesis. It means that the value of the calculated parameter is not greater than the critical value of the test (read from a relevant table); hence, the conclusion that the null hypothesis may be true.

   Errors made during verification:

   - Type I error – incorrectly rejecting the null hypothesis H_o when it is true,
   - Type II error – accepting the null hypothesis H_o when it is false.

Nowadays, statistical hypothesis testing is usually carried out using various pieces of software (e.g. *Statistica*). In this case, the procedure is limited to calculating the parameter p for a given set of data, after selecting an appropriate statistical test. The value p is then compared with the assumed value of the level of significance α.

If the calculated value p is smaller than the α value ($p < \alpha$), the null hypothesis H_o is rejected. Otherwise, the null hypothesis is not rejected.

The basic classification of a statistical test divides tests into parametric and nonparametric ones.

Parametric tests serve to verify parametric hypotheses on the distribution parameters of the examined characteristic in a parent population. Usually, they are used to test propositions concerning arithmetic mean and variance. The tests are constructed with the assumption that the CDF is known for the parent population.

Nonparametric tests are used to test various hypotheses on the goodness of fit in one population with a given theoretical distribution, the goodness of fit in two populations and the randomness of sampling.

1.8 Statistical Tests

During the processing of analytical results, various statistical tests can be used. Their description, application and inference based on these tests are presented below. Appropriate tables with critical values for individual tests are given in Appendix at the end of the book.

1.8.1 Confidence Interval Method [3]

TEST	CONFIDENCE INTERVAL METHOD
Aim	test whether a given set of results includes a result(s) with a gross error
Requirements	• set size 3–10, • unbiased series – an initially rejected uncertain result, • only one result can be rejected from a given set.
Course of action	• exclude from a set of results the result that was initially recognized as one with a gross error, • calculate the endpoints of the confidence interval for a single result based on the following formula: $$g = x_m \pm t_{crit}\sqrt{\frac{n}{n-2}}\,SD \quad (1.16)$$ where: x_m – mean for an unbiased series, SD – standard deviation for an unbiased series, n – entire size of a series, together with a uncertain result, t_{crit} – critical parameter of the t-Student test, read for $f = n - 2$ degrees of freedom – **Table A.1**.
Inference	if an uncertain result falls outside the limits of the confidence interval, it is rejected; otherwise, it is compensated for in further calculations, and the values of x_m and SD are calculated again
Requirements	• set size is 3–10, • unbiased series – an initially rejected doubtful result, • only one result can be rejected from a given set.
Course of action	• exclude from a set of results the result that was initially recognized as one with a gross error, • calculate the value of the parameter t_{calc} according to the following formula: $$t_{calc} = \frac{\left|x_i - x_m\right|}{SD} \quad (1.17)$$ where: x_i – uncertain result, x_m – mean value for the unbiased series, SD – standard deviation for the unbiased series,

- compare the value of t_{calc} with the critical value calculated according to the formula:

$$t_{crit(corr)} = t_{crit} \cdot \sqrt{\frac{n}{n-2}} \tag{1.18}$$

where:
n – entire size of a series, together with an uncertain result,
t_{crit} – critical parameter of the t-Student test, read for $f = n - 2$ degrees of freedom – **Table A.1**.

Inference if $t_{calc} \leq t_{crit(corr)}$, then the initially rejected result is included in further calculations, and x_m and SD are calculated again; otherwise, the initially rejected result is considered to have a gross error

Requirements
- set size 3–10,
- unbiased series – an initially rejected uncertain result,
- only one result can be rejected from a given set.

Course of action
- calculate the endpoints of the confidence interval for an individual result using the formula:

$$g = x_m \pm w_\alpha \cdot SD \tag{1.19}$$

where:
x_m – mean for the unbiased series,
SD – standard deviation for the unbiased series,
w_α – critical parameter determined for the number of degrees of freedom $f = n - 2$ – **Table A.2**,
n – total number of a series.

Inference if the uncertain result falls outside the endpoints of the determined confidence interval, it is rejected and x_m and SD are calculated again

Requirements
- set size >10,
- biased series.

Course of action
- calculate the endpoints of the confidence interval for an individual result using the formula:

$$g = x_m \pm k_\alpha \cdot SD \tag{1.20}$$

where:
x_m – mean for the biased series,
SD – standard deviation for the biased series,
k_α – confidence coefficient for a given level of significance α, from a normal distribution table:

for $\alpha = 0.05$ $k_\alpha = 1.65$
for $\alpha = 0.01$ $k_\alpha = 2.33$

Inference if the uncertain result(s) falls outside the endpoints of the determined confidence interval, it is rejected and x_m and SD are calculated again

Requirements
- set size >10,
- unbiased series – an initially rejected uncertain result,
- known value of the method's standard deviation.

Course of action

- calculate the endpoints of the confidence interval for an individual result using the formula:

$$g = x_m \pm k_\alpha \cdot SD_g \sqrt{\frac{n}{n-1}} \tag{1.21}$$

where:
x_m – mean for the unbiased series,
SD_g – standard deviation of the method,
k_α – confidence coefficient for a given level of significance α, from a normal distribution table

for $\alpha = 0.05$ $k_\alpha = 1.65$
for $\alpha = 0.01$ $k_\alpha = 2.33$

Inference if the uncertain result falls outside the endpoints of the determined confidence interval, it is rejected; otherwise, it is included in the series, and x_m and SD are calculated again

1.8.2 Critical Range Method [3]

TEST	CRITICAL RANGE METHOD
Aim	test whether a given set of results includes a result(s) with a gross error
Requirements	• set size >10, • known value of the method's standard deviation – SD_g.
Course of action	• calculate the value of the range result according to the formula: $R = x_{max} - x_{min}$ • calculate the value of the critical range according to the formula: $R_{crit} = z \cdot SD_g$ (1.22) where: SD_g – the standard deviation of the method, z – coefficient from the table for a given level of confidence α and n parallel measurements and f degrees of freedom – **Table A.3**.
Inference	if $R > R_{crit}$, the extremum result is rejected and the procedure is conducted anew
Requirements	• known value of the mean range for the series – R_m, • known results of k series of parallel determinations, with n determinations in each series (most often $n = 2$ or 3; $k \geq 30$).
Course of action	• calculate the value of the range for each series according to the formula: $R_i = x_{max_i} - x_{min_i}$ (1.23) • calculate the value of the critical range according to the formula: $R_{crit} = z_\alpha \cdot R_m$ (1.24) where: z_α – coefficient from a table for a given level of confidence α and n parallel measurements in a series – **Table A.4**.
Inference	if $R_i > R_{crit}$, the i-th series of the measurement results is rejected

1.8.3 Dixon's Q Test [3, 4]

TEST	DIXON'S *Q* TEST
Aim	test whether a given set of results includes a result with a gross error
Hypotheses	H_o – in the set of results, there is no result with a gross error H_1 – in the set of results, there is a result with a gross error
Requirements	• set size 3–10, • test whether a given set of results includes a result with a gross error.
Course of action	• order the results in a non-decreasing sequence: $x_1 \ldots x_n$, • calculate the value of the range R according to the formula: • $R = x_n - x_1$, • calculate the value of parameters Q_1 and Q_n according to the formulas: $$Q_1 = \frac{x_2 - x_1}{R} \qquad Q_n = \frac{x_n - x_{n-1}}{R} \tag{1.25}$$ • compare the obtained values with the critical value Q_{crit} – **Table A.5** read for the selected level of significance α and the number of degrees of freedom $f = n$.
Inference	if one of the calculated parameters exceeds the critical value Q_{crit}, then the result from which it was calculated (x_n or x_1) should be rejected as a result with a gross error and only then should x_m and SD be calculated

In some studies [1], the authors use a certain type of Dixon's Q test that makes it possible to test a series comprising up to 40 results.

TEST	DIXON'S *Q* TEST
Aim	test whether a given set of results includes a result with a gross error
Hypotheses	H_o – in the set of results, there is no result with a gross error H_1 – in the set of results, there is a result with a gross error
Requirements	• set size 3–7, • test whether a given set of results includes a result with a gross error.
Course of action	• order the results as a non-decreasing sequence: $x_1 \ldots x_n$, • calculate the value of the range R according to the formula: $$R = x_n - x_1$$ • calculate the value of parameters Q_1 and Q_n according to the formulas: $$Q_1 = \frac{x_2 - x_1}{R} \qquad Q_n = \frac{x_n - x_{n-1}}{R} \tag{1.26}$$ • compare the obtained values with the critical value Q_{crit} – **Table A.6** read for the selected level of significance α and the number of degrees of freedom $f = n$.
Requirements	• set size 8–12, • test whether a given set of results includes a result with a gross error.

Course of action
- order the results as a non-decreasing sequence: $x_1 \ldots x_n$,
- calculate the value of parameters Q_1 and Q_n according to the formulas:

$$Q_1 = \frac{x_2 - x_1}{x_{n-1} - x_1} \qquad Q_n = \frac{x_n - x_{n-1}}{x_n - x_2} \tag{1.27}$$

- compare the obtained values with the critical value Q_{crit} – **Table A.6** read for the selected level of significance α and the number of degrees of freedom $f = n$.

Requirements
- set size >12,
- test whether a given set of results includes a result with a gross error.

Course of action
- order the results as a non-decreasing sequence: $x_1 \ldots x_n$,
- calculate the value of parameters Q_1 and Q_n according to the formulas:

$$Q_1 = \frac{x_3 - x_1}{x_{n-2} - x_1} \qquad Q_n = \frac{x_n - x_{n-2}}{x_n - x_3} \tag{1.28}$$

- compare the obtained values with the critical value Q_{crit} – **Table A.6** read for the selected level of significance α and the number of degrees of freedom $f = n$.

Inference if one of the calculated parameters exceeds the critical value Q_{crit}, then the result from which it was calculated (x_n or x_1) should be rejected as a result with a gross error and only then should x_m and SD be calculated

1.8.4 Chi Square (χ^2) Test [3]

TEST	CHI SQUARE (χ^2) TEST
Aim	test if the variance for a given series of results is different from the set value
Hypotheses	H_0 – the variance calculated for the series of results is not different from the set value in a statistically significant manner H_1 – the variance calculated for the series of results is different from the set value in a statistically significant manner
Requirements	• normal distribution of results in a series
Course of action	• calculate the standard deviation for the series of results, • calculate the chi square test parameter χ^2 according to the formula:

$$\chi^2 = \frac{n \cdot SD^2}{SD_o^2} \tag{1.29}$$

where:
SD – the standard deviation calculated for the set of results,
SD_o – the set value of the standard deviation,
n – the number of results in an investigated set.

- compare the calculated value χ^2 with the critical value χ^2_{crit} for the assumed level of significance α and the calculated number of degrees of freedom $f = n - 1$ – **Table A.7**.

Inference

- if the calculated value χ^2 does not exceed the critical value ($\chi^2 \leq \chi^2_{crit}$), then it may be inferred that the calculated value of the standard deviation does not differ in a statistically significant manner from the set value – acceptance of hypothesis H_o,
- if the calculated value χ^2 is greater than the critical value read from the tables ($\chi^2 > \chi^2_{crit}$), then it may be inferred that the compared values of the standard deviation differ in a statistically significant manner – rejection of the hypothesis H_o.

1.8.5 Snedecor's F *Test [3–5]*

TEST	SNEDECOR'S *F* TEST
Aim	compare the standard deviations (variances) for two sets of results
Hypotheses	H_o – the variances calculated for the compared series of results do not differ in a statistically significant manner H_1 – the variances calculated for the compared series of results differ in a statistically significant manner
Requirements	• normal distributions of results in a series
Course of action	• calculate the standard deviations for the compared series of results, • calculate Snedecor's *F* test parameter according to the formula:

$$F = \frac{SD_1^2}{SD_2^2} \tag{1.30}$$

where:
SD_1, SD_2 – standard deviations for the two sets of results,

NOTE: The value of the expression should be constructed in such a way so that the numerator is greater than the denominator – the value *F* should always be greater than 1

- compare the calculated value with the critical value of the assumed level of significance α and the calculated number of freedom degrees f_1 and f_2 (where $f_1 = n_1 - 1$ and $f_2 = n_2 - 1$) – **Table A.8**.

Inference

- if the calculated value *F* does not exceed the critical value ($F \leq F_{crit}$), then it may be inferred that the calculated values for the standard deviation do not differ in a statistically significant manner – acceptance of the hypothesis H_o,
- if the calculated value *F* is greater than the critical value read from the tables ($F > F_{crit}$), then it may be inferred that the compared values for the standard deviation differ in a statistically significant manner – rejection of the hypothesis H_o.

1.8.6 Hartley's F_{max} *Test [3]*

TEST	HARTLEY'S F_{max} TEST
Aim	compare the standard deviations (variances) for many sets of results
Hypotheses	H_0 – the variances calculated for the compared series of results do not differ in a statistically significant manner H_1 – the variances calculated for the compared series of results differ in a statistically significant manner
Requirements	• normal distributions of results in a series, • numbers of results in each series of the sets greater than 2, • set sizes are identical, • the number of series not greater than 11.
Course of action	• calculate the standard deviations for the compared series of results, • calculate the value of the F_{max} test parameter according to the formula: $$F_{max} = \frac{SD_{max}^2}{SD_{min}^2} \quad (1.31)$$ where: SD_{max}, SD_{min} – the greatest and smallest value from the calculated standard deviations for the sets of results. In the case of different values of results in the series, use CV instead of SD according to the formula: $$F_{max} = \frac{CV_{max}^2}{CV_{min}^2} \quad (1.31a)$$ • compare the calculated value with the critical value of the parameter for the assumed level of significance α, the calculated number of degrees of freedom $f = n - 1$, and the number of the compared series k – **Table A.9**.
Inference	• if the calculated value F_{max} does not exceed the critical value ($F_{max} \leq F_{max_\alpha}$), then it may be inferred that calculated standard deviations do not differ in a statistically significant manner – acceptance of the hypothesis H_0, • if the calculated value F_{max} is greater than the critical value read from the tables ($F_{max} > F_{max_\alpha}$), then it may be inferred that the compared standard deviations differ in a statistically significant manner – rejection of the hypothesis H_0.

1.8.7 Bartlett's Test [3]

TEST	BARTLETT'S TEST
Aim	compare the standard deviations (variances) for many sets of results
Hypotheses	H_0 – the variances calculated for the compared series of results do not differ in a statistically significant manner H_1 – the variances calculated for the compared series of results differ in a statistically significant manner
Requirements	• the number of results in each series of the sets is greater than 2

Course of action

- calculate the standard deviation for the compared series of results,
- calculate the value of a Q test parameter according to the formula:

$$Q = \frac{2.303}{c}\left[(n-k)\log\left(\overline{SD_o^2}\right) - \sum_{i=1}^{k}(n_i - 1)\log\left(SD_i^2\right)\right] \tag{1.32}$$

in which:

$$c = 1 + \frac{1}{3(k-1)}\left(\sum_{i=1}^{k}\frac{1}{n_i - 1} - \frac{1}{n-k}\right) \tag{1.33}$$

$$\overline{SD_o^2} = \frac{1}{n-k}\sum_{n=1}^{k} SD_i^2\,(n_i - 1) \tag{1.34}$$

where:
n – the total number of parallel determinations,
k – the number of the compared method (series),
n_i – the number of parallel determinations in a given series,
SD_i – the standard deviation for the series i.

- compare the calculated value with the critical value of χ^2_{crit} parameter for the assumed level of significance α and the calculated number of degrees of freedom $f = k - 1$ – **Table A.7**.

Inference

- if the calculated value Q does not exceed the critical value ($Q \le \chi^2_{crit}$), then it may be inferred that the calculated standard deviations do not differ in a statistically significant manner – acceptance of the hypothesis H_o,
- if the calculated value Q is greater than the critical value read from the tables ($Q > \chi^2_{crit}$), then it may be inferred that the compared standard deviations differ in a statistically significant manner – rejection of the hypothesis H_o.

1.8.8 Morgan's Test [3]

TEST	MORGAN'S TEST
Aim	compare standard deviations (variances) for two sets of dependent (correlated) results
Hypotheses	H_o – the variances calculated for the compared series of results do not differ in a statistically significant manner H_1 – the variances calculated for the compared series of results differ in a statistically significant manner
Requirements	• number of results in each series of the sets is greater than 2

Course of action

- calculate the standard deviations for the compared series of results,
- calculate the regression coefficient r according to the formula:

$$r = \frac{k\sum_{i=1}^{k} x_{1i}x_{2i} - \sum_{i=1}^{k} x_{1i}\sum_{i=1}^{k} x_{2i}}{\sqrt{\left[k\sum_{i=1}^{k} x_{1i}^2 - \left(\sum_{i=1}^{k} x_{1i}\right)^2\right]\left[k\sum_{i=1}^{k} x_{2i}^2 - \left(\sum_{i=1}^{k} x_{2i}\right)^2\right]}} \tag{1.35}$$

- calculate the value of test L parameter according to the formula:

$$L = \frac{4SD_1^2 SD_2^2\left(1-r^2\right)}{\left(SD_1^2 + SD_2^2\right)^2 - 4r^2 SD_1^2 SD_2^2} \tag{1.36}$$

- calculate the value of parameter t according to the formula:

$$t = \sqrt{\frac{(1-L)(k-2)}{L}} \tag{1.37}$$

where:
k – the number of pairs of results,
x_{1i}, x_{2i} – individual values of results for the compared sets.

- compare the calculated value t with the critical value t_{crit}, a parameter for the assumed level of significance α the calculated number of degrees of freedom $f = k - 2$ – **Table A.1**.

Inference

- if the calculated value t does not exceed the critical value t_{crit}, so that the relation $t \le t_{crit}$ is satisfied, then it may be inferred that the calculated standard deviations do not differ in a statistically significant manner – acceptance of hypothesis H_o,
- if the calculated value t is greater than the critical value read from the tables ($t > t_{crit}$), then it may be inferred that the compared standard deviations differ in a statistically significant manner – rejection of the hypothesis H_o.

1.8.9 Student's t *Test [3, 4]*

TEST	STUDENT'S *t* TEST
Aim	compare means for two series (sets) of results
Hypotheses	H_o – the calculated means for the compared series of results do not differ in a statistically significant manner H_1 – the calculated means for the compared series of results differ in a statistically significant manner
Requirements	• normal distributions of results in a series, • number of results in each series of the sets greater than 2, • insignificant variance differences for the compared sets of results – Snedecor's F test, Section 1.8.5.

Course of action

- calculate the means and standard deviations for the series of results,
- calculate the Student's t test parameter according to the equation:

$$t = \frac{|x_{1m} - x_{2m}|}{\sqrt{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}} \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}} \qquad (1.38)$$

where:

x_{1m}, x_{2m} – the means calculated for the two compared sets of results,
SD_1, SD_2 – the standard deviations for the sets of results.

- compare the calculated value with the critical value of a parameter for the assumed level of significance α and the calculated number of degrees of freedom $f = n_1 + n_2 - 2$ – **Table A.1**.

Inference

- if the value t does not exceed the critical value t_{crit}, ($t \leq t_{crit}$), then it may be inferred that the obtained means do not differ in a statistically significant manner – acceptance of the hypothesis H_o,
- if the calculated value t is greater than the critical value read from the tables ($t > t_{crit}$), then it is inferred that the compared means differ in a statistically significant manner – rejection of the hypothesis H_o.

TEST	STUDENT'S t TEST
Aim	compare the mean with the assumed value
Hypotheses	H_o – the calculated mean does not differ in a statistically significant manner from the assumed value H_1 – the calculated mean differs in a statistically significant manner from the assumed value
Requirements	• normal distribution of results in a series, • the number of results in a series of sets is greater than 2.
Course of action	• calculate the mean and standard deviation for the series of results, • calculate the Student's t test parameter according to the equation:

$$t = \frac{|x_m - \mu|}{SD} \sqrt{n} \qquad (1.39)$$

where:

x_m, – the mean calculated for the set of results,
μ – the reference (e.g. certified value),
SD – the unit of deviation for example: the standard deviation of the set of results which the mean was calculated based on,
n – the number of results.

- compare the calculated value with the critical value of a parameter, for the assumed level of significance α, the calculated number of degrees of freedom $f = n - 1$ – **Table A.1**.

Inference

- if the value t does not exceed the critical value t_{crit}, ($t \leq t_{crit}$), then it may be inferred that the obtained mean is not different from the set value in a statistically significant manner – acceptance of the hypothesis H_o,
- if the calculated value t is greater than the critical value read from the tables ($t > t_{crit}$), it is inferred that the mean is different from the set value in a statistically significant manner – rejection of the hypothesis H_o.

1.8.10 Cochran-Cox C Test [3]

TEST	COCHRAN-COX *C* TEST
Aim	compare the means for the series of sets of results, for which the standard deviations (variances) differ in a statistically significant manner
Hypotheses	H_o – the calculated means for the compared series of results do not differ in a statistically significant manner H_1 – the calculated means for the compared series of results differ in a statistically significant manner
Requirements	• normal distribution of results in a series, • the number of results in a series of sets is greater than 2.
Course of action	• calculate the means and standard deviations for the compared series of results, • calculate the value of a parameter *C* according to the formula:

$$C = \frac{\left| x_{1m} - x_{2m} \right|}{\sqrt{z_1 + z_2}} \tag{1.40}$$

in which:

$$z_1 = \frac{SD_1^2}{n_1 - 1}, \quad \text{and} \quad z_2 = \frac{SD_2^2}{n_2 - 1} \tag{1.41}$$

where:

x_{1m}, x_{2m} – the means calculated for the two compared sets of results,
SD_1, SD_2 – the standard deviations for the sets of results.

- calculate the critical value of the parameter C (C_{crit}) according to the formula:

$$C_{crit} = \frac{z_1 t_1 + z_2 t_2}{z_1 + z_2} \tag{1.42}$$

where:

t_1 and t_2 – the critical values read from the tables of the Student's t distribution (**Table A.1**) respectively for $f_1 = n_1 - 1$ and $f_2 = n_2 - 1$, the number of degrees of freedom and the level of significance α

Inference	• compare the calculated value C with the calculated critical value C_{crit} • if the value C does not exceed the critical value C_{crit}, ($C \leq C_{crit}$), then it may be inferred that the obtained mean values do not differ from one another in a statistically significant manner – acceptance of the hypothesis H_o, • if the calculated value C is greater than the calculated critical value ($C > C_{crit}$), then it is inferred that the obtained means differ from one another in a statistically significant manner – rejection of the hypothesis H_o.

1.8.11 Aspin-Welch Test [3]

TEST	ASPIN-WELCH TEST
Aim	compare the means for the series of sets of results for which the standard deviations (variances) differ in a statistically significant manner
Hypotheses	H_o – calculated means for the compared series of results do not differ in a statistically significant manner H_1 – calculated means for the compared series of results differ in a statistically significant manner
Requirements	• normal distribution of results in a series, • the number of results in a series of sets is greater than 6.
Course of action	• calculate the means and standard deviations for the compared series of results, • calculate the values of expressions described using the following equations:

$$\nu = \frac{\left|x_{1m} - x_{2m}\right|}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}} \tag{1.43}$$

$$c = \frac{\frac{SD_1^2}{n_1}}{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}} \tag{1.44}$$

in which:

$$\frac{SD_1^2}{n_1} < \frac{SD_2^2}{n_2} \tag{1.45}$$

where:

x_{1m}, x_{2m} – the means calculated for the two compared sets of results,
SD_1, SD_2 – the standard deviations for the sets of results.

- compare the calculated value ν with the critical value ν_o for the corresponding level of significance α, the number of degrees of freedom $f_1 = n_1 - 1$, $f_2 = n_2 - 1$ and the calculated values of c, and thus $\nu_o(\alpha, f_1, f_2, c)$ – **Table A.10**.

Inference	• if the value ν does not exceed the critical value ν_o, ($\nu \le \nu_o$), then it may be inferred that the obtained means do not differ from one another in a statistically significant manner – acceptance of the hypothesis H_o, • if the calculated value ν is greater than the calculated critical value ($\nu > \nu_0$), it is inferred that the obtained means differ from one another in a statistically significant manner – rejection of the hypothesis H_o.

1.8.12 Cochran's Test [6]

TEST	COCHRAN'S TEST
Aim	detection of outliers in a given set – intralaboratory variability test one-sided test for outliers – the criterion of the test examines only the greatest standard deviations
Requirements	• the number of results in a series (set) greater than or equal to 2, but only when the number of compared laboratories is greater than 2, • sets of results (series) with the same numbers, • it is recommended to apply the tests before the *Grubbs'* test – Section 1.8.13.
Course of action	• calculate the standard deviations for each of the compared sets of results, • calculate the value of parameter C using the formula: $$C = \frac{SD_{max}^2}{\sum_{i=1}^{p} SD_i^2} \quad (1.46)$$ where: SD_{max} – maximum standard deviation in the investigated set (among the investigated laboratories), SD_i – the standard deviation for a given series (data from a laboratory), p – the number of standard deviations (the number of compared laboratories). • compare the calculated value C with the critical value for a given value n, the number of results in a series and p, the number of laboratories – **Table A.11**.
Inference	• if the value of the calculated test parameter is less than or equal to the critical value corresponding to the level of significance $\alpha = 0.05$, then the investigated result is considered to be correct, • if the numerical value of a respective test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.05$ and less than or equal to the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated result is an uncertain value, • if the value of the test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated result is considered to be an outlier.

1.8.13 Grubbs' Test [6, 7]

TEST	GRUBBS' TEST
Aim	detect outliers in a given set – interlaboratory variability test
Requirements	• the number of results in a series (set) is greater than or equal to 2, but only when the number of compared laboratories is greater than 2, • the same number of results in the sets (series) of results, • it is recommended to apply this test before *Cochran's* test – Section 1.8.12, • with a single use, it enables the detection of one outlier; thus, it should be repeated until no outliers are observed in the remaining results.

Course of action

- calculate the standard deviation for the set of results,
- order the set of data x_i for $i = 1, 2, ..., p$ in an increasing sequence,
- calculate the value of parameter G_p according to the relation:

$$G_p = \frac{\left(x_p - x_m\right)}{SD} \tag{1.47}$$

where:
x_p – the value in the set of results considered to be an outlier,
x_m – the mean,
SD – the standard deviation.

- compare the calculated value G_p with the critical value for a given value p, the number of laboratories – **Table A.12**.

Inference

- if the value of the calculated test parameter is less than or equal to the critical value corresponding to the level of significance $\alpha = 0.05$, then the investigated result is considered to be correct,
- if the numerical value of a corresponding test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.05$, and less than or equal to the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated result is an uncertain value,
- if the value of the test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated result is considered to be an outlier; after rejection of this value from the set of results, the test for the series of $p - 1$ results may be conducted again, and the course of action should be continued until there are no more outliers in the set of results.

TEST	GRUBBS' TEST
Aim	detect outliers in a given set – interlaboratory variability test

Requirements

- the number of results in a series (set) is greater than or equal to 2, but only when the number of compared laboratories is greater than 2,
- the same number of results in the sets of results (series)
- it is recommended to apply this test before *Cochran's* test – Section 1.8.12,
- in a given course of action, two (the greatest or the smallest) results may be rejected from the set of results.

Course of action

- order the set of data x_i for $i = 1, 2, ..., p$ in an increasing sequence, calculate the values of parameter SD_o according to the equation:

$$SD_o^2 = \sum_{i=1}^{p}\left(x_i - x_m\right)^2 \tag{1.48}$$

- calculate the values of parameters $x_{m(p-1,p)}$ when testing two of the highest results or $x_{m(1,2)}$ two of the lowest results, according to the equations:

$$x_{m(p-1,p)} = \frac{1}{p-1}\sum_{i=1}^{p-2} x_i, \text{ or } x_{m(1,2)} = \frac{1}{p-1}\sum_{i=3}^{p} x_i \tag{1.49}$$

- calculate the values of respective parameters: $SD_{(p-1,p)}$ or $SD_{(1,2)}$ according to the equations:

$$SD^2_{(p-1,p)} = \sum_{i=1}^{p-2}\left(x_i - x_{m(p-1,p)}\right)^2, \quad \text{or} \quad SD^2_{(2,1)} = \sum_{i=3}^{p}\left(x_i - x_{m(1,2)}\right)^2 \tag{1.50}$$

- calculate the value of parameter G according to the equations:

$$G = \frac{SD^2_{(p-1,p)}}{SD^2_o}, \quad \text{or} \quad G = \frac{SD^2_{(1,2)}}{SD^2_o} \tag{1.51}$$

- compare the calculated value of G with the critical value for a given value p, the number of laboratories – **Table A.12**.

Inference

- if the value of the calculated test parameter is less than or equal to the critical value corresponding to the level of significance $\alpha = 0.05$, then the investigated results are considered to be correct,
- if the numerical value of a corresponding test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.05$ and less than or equal to the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated results are uncertain,
- if the value of a test parameter is greater than the critical value corresponding to the level of significance $\alpha = 0.01$, then the investigated results are considered to be outliers; after rejection of these values from the set of results, the test for the series of $p - 2$ results may conducted again, and the course of action should be continued until there are no more outliers in the set of results.

1.8.14 Hampel's Test

The Hampel's test by some authors is called *Huber's* test [8, 9].

TEST	HAMPEL'S TEST
Aim	detect outliers in a given set
Requirements	• the number of results in a series (set) is greater than 2
Course of action	• order the values in an increasing sequence, • calculate the median Me for all the results x_i, where x_i includes the interval from x_1 to x_n, • calculate the deviations of r_i from the median for each result using the formula: $r_i = \left(x_i - Me\right)$ (1.52) • calculate the absolute values $\lvert r_i \rvert$, • order the values of $\lvert r_i \rvert$ in an increasing sequence, • calculate the median deviations $Me_{\lvert r_i \rvert}$, • compare the values of $\lvert r_i \rvert$ with $4.5 \cdot Me_{\lvert r_i \rvert}$.
Inference	if the following condition is satisfied $\lvert r_i \rvert \geq 4.5 \cdot Me_{\lvert r_i \rvert}$ (1.53) then the result x_i is considered to be an outlier.

1.8.15 Z-Score [10, 11]

TEST	*Z*-SCORE
Aim	detect uncertain results and outliers applied during the processing of results of interlaboratory comparisons
Requirements	• the number of results in a series (set) greater than 2
Course of action	• calculate the *Z* score using the formula:

$$Z = \frac{x_{lab} - x_{ref}}{SD} \tag{1.54}$$

where:

x_{lab} – result obtained by a given laboratory,
x_{ref} – the assumed value/the reference value,
SD – the deviation unit:

- the standard deviation calculated using all the values in a set,
- the modified standard deviation calculated using the relation:

$$SD_{mod} = \sqrt{SD^2 + u^2_{(x_{ref})}} \tag{1.55}$$

where:

$u_{(x_{ref})}$ – standard uncertainty of the accepted value/the reference value,

- combined standard uncertainty calculated using the relation:

$$u = \sqrt{u^2_{(x_{lab})} + u^2_{(x_{ref})}} \tag{1.56}$$

where:

$u_{(x_{lab})}$ – standard uncertainty of a value obtained by a given laboratory.

Inference	• if $\lvert Z \rvert \le 2$, a result is considered to be satisfactory, • if $2 < \lvert Z \rvert < 3$, a result is considered to be uncertain, • if $\lvert Z \rvert \ge 3$, a result is considered to be unsatisfactory.

1.8.16 E_n-Score [10, 11]

TEST	E_N-SCORE
Aim	estimation of results of interlaboratory comparisons
Requirements	• the number of results in a series (set) is greater than 2
Course of action	• calculate E_n score using the formula:

$$E_n = \frac{x_{lab} - x_{ref}}{\sqrt{u^2_{(x_{lab})} + u^2_{(x_{ref})}}} \tag{1.57}$$

where:

x_{lab} – the value obtained by a given laboratory,
x_{ref} – the reference value,
$u_{(x_{lab})}$ – the combined standard uncertainty result obtained by a given laboratory,
$u_{(x_{ref})}$ – the combined standard uncertainty of the reference values.

Inference
- if $|E_n| \leq 1$, the estimation is satisfactory,
- if $|E_n| > 1$, the estimation is unsatisfactory.

1.8.17 Mandel's Test [6, 12, 13]

TEST	MANDEL'S *h* TEST
Aim	determine the interlaboratory traceability of results
Requirements	• the number of results in a series (set) is greater than 2
Course of action	• calculate the means x_{mi} for each series of results for each laboratory, • calculate the mean for results from a given series according to the formula:

$$\overline{x_m} = \frac{\sum_{i=1}^{p} n_i \cdot x_{mi}}{\sum_{i=1}^{p} n_i} \tag{1.58}$$

where:
n_i – the number of results for a given series obtained by a given laboratory,
p – the number of laboratories.

- calculate the values of parameter h_i for a given series and for a given laboratory, according to the formula:

$$h_i = \frac{x_{mi} - \overline{x_m}}{\sqrt{\frac{1}{(p-1)} \sum_{i=1}^{p} (x_{mi} - \overline{x_m})^2}} \tag{1.59}$$

- make a graph of the values of parameter h_i for each series in the sequence of laboratories,
- on the graph of the values of parameter h, mark the horizontal lines to test the data's configuration, corresponding to the Mandel h coefficients for a given level of significance ($\alpha = 0.01$ or 0.05) — **Table A.13a** or **A.13b**.

Inference The value of parameter h_i bigger than h value need to be checked from analytical viewpoint.

TEST	MANDEL'S *k* TEST
Aim	determine the interlaboratory traceability of results
Requirements	• the number of results in a series (set) is greater than 2

Course of action

- calculate the standard deviations SD_i for each series of results for each laboratory,
- calculate the values of parameter k_i for a given series and for a given laboratory, according to the formula:

$$k_i = \frac{SD_i\sqrt{p}}{\sqrt{\sum SD_i^2}} \tag{1.60}$$

- make a graph of the values of parameter k_i for each series in the sequence of laboratories,
- on the graph of the values of parameter k, mark the horizontal lines to test the data's configuration, corresponding to the Mandel k coefficients for a given level of significance ($\alpha = 0.01$ or 0.05) – **Table A.13a** or **A.13b**.

Inference The value of parameter k_i bigger than k value needs to be checked from analytical viewpoint.

1.8.18 Kolmogorov-Smirnov Test [2, 14]

TEST	KOLMOGOROV-SMIRNOV TEST
Aim	compare the distribution of two series of results
Requirements	• two series of results
Course of action	• calculate the empirical distribution functions for each series of results, • calculate the values of parameter λ_n, according to the formula:

$$\lambda_n = \sqrt{\frac{n_1 n_2}{n_1 + n_2}} D_{n_1 n_2} \tag{1.61}$$

where:
n_1, n_2 – the number of results for a given series,
$D_{n_1 n_2}$ – the greatest value of differences between empirical distribution functions.

- compare the λ_n value with critical value – λ_α for a given level of significance – **Table A.14**.

Inference

- if the value λ_n does not exceed the critical value λ_α, ($\lambda_n \leq \lambda_\alpha$), then it may be inferred that there are no statistically significant differences in distribution functions for both compared series,
- if the value λ_n does exceed the critical value λ_α, ($\lambda_n > \lambda_\alpha$), then it may be inferred that there are statistically significant differences in distribution functions for both compared series.

1.9 Linear Regression

Linear correlation is the most frequent correlation used in analytical chemistry. A decisive majority of analytical measurements uses the

calibration stage, in which the values of the output signal are assigned to corresponding values of analyte concentration. To determine the functional dependency that connects the output signal with analyte concentration, a linear regression method is applied. It is also applied in determining some of the validation parameters of the analytical procedure, such as:

- accuracy – through the determination of systematic errors,
- linearity,
- limits of detection.

Therefore, we present below the course of action for the linear regression method, together with a presentation of the determination method for the calibration chart parameters.

The equation of the linear regression is as follows:

$$y = b \cdot x + a \tag{1.62}$$

where:

y – dependent variable (output signal of the measuring instrument),
x – independent variable (concentration of the determined analyte),
a – intercept,
b – slope.

The following regression parameters are calculated [3]:

- slope – b:

$$b = \frac{\sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i - n \sum_{i=1}^{n} x_i y_i}{\left(\sum_{i=1}^{n} x_i \right)^2 - n \sum_{i=1}^{n} x_i^2} \tag{1.63}$$

- intercept value – a:

$$a = \frac{\sum_{i=1}^{n} y_i - b \sum_{i=1}^{n} x_i}{n} \tag{1.64}$$

- regression coefficient – r:

$$r = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\sqrt{\left[n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2\right] \cdot \left[n\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2\right]}} \tag{1.65}$$

- values of standard deviations for:
  - slope – SD_b:

$$SD_b = \frac{SD_{xy}}{\sqrt{\sum_{i=1}^{n} x_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} x_i\right)^2}} \tag{1.66}$$

  - intercept – SD_a:

$$SD_a = SD_{xy}\sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2}} \tag{1.67}$$

  - residuals – SD_{xy}:

$$SD_{xy} = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - Y_i\right)^2}{n-2}} \tag{1.68}$$

where:

n – the number of independent determination results for the standard solution samples from which the calibration curve has been determined,
y_i – the value determined experimentally,
Y_i – the value calculated from the determined regression equation.

1.10 Significant Digits. Rules of Rounding

A problem with correct notation of the measurement results is usually associated with issues related to significant digits and the rules of rounding.

Significant digits in the decimal notation of a given number are all the digits without initial zeros. In order to determine how many

significant digits there are in a number, the number should be "read" from left to right until reaching the first digit that is not zero. That digit and all the subsequent digits are called significant. In the example below, the significant digits are underlined:

$$\underline{230.546}$$
$$0.00\underline{10823}$$
$$\underline{20.1200}$$
$$\underline{507.80}$$
$$0.\underline{63}\times 10^{4}$$
$$\underline{34.70}$$

Calculations very often use values with different numbers of significant digits and different numbers of digits after the decimal point. A value obtained from a calculation(s) should be recorded in an appropriate way, strictly dependent on the notation of the values applied in the calculation(s).

After addition or subtraction, the value of a result should be presented with the same number of digits after the decimal point as the value with the fewest number of digits after the decimal point.

For example, if a result is the sum of numbers:

$$\begin{aligned} &11.23\\ &15.2113\\ &0.123\\ &349.2 \end{aligned}$$

then it should be presented with one digit after the decimal point:

$$375.8$$

For multiplication and division, the number of significant digits in a result should be the same as in the value with the fewest significant digits.

If a result is a product of the following numbers:

$$\begin{aligned} &11.23\\ &15.2113\\ &0.123\\ &349.2 \end{aligned}$$

Then, it should be presented with three significant digits:

$$73.4 \cdot 10^2$$

It must be remembered that the number of significant digits given in the value of a result is strictly dependent on the calculated uncertainty value (see Chapter 5). The notation of the determination requires presentation of the uncertainty value with maximum two significant digits and a result with the same precision (same number of figures after the decimal point) as the uncertainty value. This requirement frequently makes it necessary to round the obtained values down to the appropriate number of digits.

References

1. Dobecki M. (ed), Zapewnienie jakości analiz chemicznych, Instytut Medycyny Pracy im. Prof. J. Nofera, Łódź 2004 (*in Polish*).
2. Koronacki J., and Mielniczuk J., Statystyka dla studentów kierunków technicznych i przyrodniczych, Warsaw, WNT, 2018 (*in Polish*).
3. Bożyk Z., and Rudzki W., Metody statystyczne w badaniu jakości produktów żywnościowych i chemicznych, Warsaw, WNT, 1977 (*in Polish*).
4. Kozłowski E., Statystyczne kryteria oceny wyników i metod analitycznych w: Bobrański B.: Analiza ilościowa związków organicznych, Warsaw, PWN, 1979 (*in Polish*).
5. Czerminski J.B., Iwasiewicz A., Paszek Z., and Sikorski A., Statistical Methods in Applied Chemistry, Elsevier, 1990.
6. ISO 5725-2:2019. Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.
7. Doerffel K., Statystyka dla chemików analityków, Warsaw, WNT, 1989 (*in Polish*).
8. Davies P. L., Statistical evaluation of interlaboratory tests, *Fresenius Z. Anal. Chem.*, 331, 513–519, 1988.
9. Linsinger T.P.J., Kandel W., Krska R., and Grasserbauer M., The influence of different evaluation techniques on the results of interlaboratory comparisons, *Accred. Qual. Assur.*, 3, 322–327, 1998.
10. Cortez L., Use of LRM in Quality Control: Interlaboratory Testing – EC Growth Projects TRAP-LRM/TRAP-NAS, 2001.
11. ISO/IEC 17043:2023 Conformity assessment—General requirements for the competence of proficiency testing providers.
12. Van Dyck K., Robberecht H., Van Cauwenbergh R., Deelstra H., Arnaud J., Willemyns L., Benijts F., Centeno J.A., Taylor H., Soares M.E., Bastos M.L., Ferreira M.A., D'Haese P.C., Lamberts L.V.,

Hoenig M., Knapp G., Lugowski S.J., Moens L., Riondato J., Van Grieken R., Claes M., Verheyen R., Clement L., and Uytterhoeven M., Spectrometric determination of silicon in food and biological samples: an interlaboratory trial, *J. Anal. At. Spectrom.*, 15, 735–743, 2000.

13. Cools N., Delanote V., Scheldeman X., Quataert P., De Vos B., and Roskams P., Quality assurance and quality control in forest soil analyses: a comparison between European soil laboratories, *Accred. Qual. Assur.*, 9, 688–694, 2004.
14. Achnazarowa S.Ł., and Kafarow W.W., Optymalizacja eksperymentu w chemii i technologii chemicznej, Warsaw, WNT, 1982 (*in Polish*).

2
Quality of Analytical Results

2.1 Definitions [1–3]

Quality – the realization of specific requirements (which include the standards established by the quality control system in addition to accepted in-house requirements).

Analytical quality – consistency of the obtained results (chemical analysis) with the accepted assumptions. The quality of information can be divided into components: quality of results, quality of the process, quality of the instruments and quality of the work and organization.

Quality control – a complex system of actions to obtain measurement (determination results) with the required quality level. A program of quality control includes:

- assuring a suitable level of staff qualifications,
- assuring the proper calibration of instruments and laboratory equipment,
- good laboratory practice (GLP),
- standard procedures.

2.2 Introduction

The past decade or so was undoubtedly the period of "information hunger". Access to a variety of information sources facilitates decision-making not only in politics but also in the economy and technology (related to control over the processes of manufacturing consumer goods). A new type of market arose, where information is bought and sold.

Analytical data on the researched material objects are a specific kind of information. This information is not usually obtained through an analysis of the whole object but is based on the analyses of appropriate

samples. Therefore, samples have to be collected in such a way that the most important criterion – that is, representativeness – is met.

To satisfy the growing demand for analytical data, more and more intense research is taking place with the aim of developing new methodologies and devices so that the analytical results are a source of as much information as possible, which – in other words – are characterized by the greatest information capacity possible.

Measurement results must be reliable, which means they must accurately (both truly and precisely) reflect the real content (amount) of analytes in a sample that is representative of the material object under research. This leads to the conclusion that all developments in analytical chemistry are derived from the desire to obtain in-depth analytical data [4].

The notion of reliability is closely associated with the notion of quality. It is the quality of a result, together with its control and assurance, that determines and confirms its reliability. In analytics, the notions of quality have a specific meaning.

Results of analytical measurements are a type of a product of the chemical analyst's work.

Both manufactured products and analytical results must have an appropriate quality. In addition, the quality of analytical measurements appears to have its own accumulative requirement: the quality of every product is a result of comparison of the obtained value with the reference value, expected or the standard one. For the obtained result to be comparable (authoritative, reliable) to the reference value, its (high) quality must be documented and maintained. The quality of results of analytical measurements must be assured in the first place to draw conclusions about the quality of the examined products.

2.3 Quality Assurance System

One of basic trends in the recent development of analytical chemistry is determination of lower and lower concentrations of analytes in samples with a complex matrix. The need for a uniform and defined control system, of estimation and assuring the quality of analytical results, is a consequence of the following trends in analytics:

- decrease in the concentrations of analytes,
- increase in the complexity of the matrix composition of the sample,

- introduction of new notions associated with the application of metrology principles in analytics,
- necessity of traceability documentation and estimating uncertainty as requisite parameters of an analytical result,
- globalization and the associated necessity of comparing results in different laboratories.

This task poses a great challenge for analysts and draws attention to quality assurance and quality control (QA/QC) of the obtained results. The system of quality estimation usually includes the following elements:

- tracking and estimating the precision of obtained results by periodic analysis of test samples,
- estimation of accuracy by:
  - analyses of certified reference samples,
  - comparison of obtained results with results obtained for the same sample using the reference method,
  - sample analyses after the addition of a standard,
  - comparative interlaboratory (intercomparison) exercises,
- control charts,
- suitable audit system.

At present, there are three systems of quality assurance in analytical laboratories [5]:

- good laboratory practice (*GLP*),
- accreditation of a laboratory according to ISO Guide 17025 or EN 45001,
- certification according to norms ISO of series 9000.

The selection of the quality system, introduced by a given laboratory, is in principle voluntary, although increasing attention is paid to the procedures of accreditation [6].

The problem relating to quality assurance and control of measurement results is primarily associated with the insufficient amount of information concerning instruments used in the process and their application. These are first of all statistical instruments based on metrology.

Quality assurance of analytical measurement results is the system comprising five interdependent elements [7]:

- assurance of measuring traceability of the obtained results,
- estimation of uncertainty obtained results of measurement,

- using of certified reference materials,
- participation in various interlaboratory comparisons,
- validation of the applied analytical procedures.

Only when the aforementioned tools are used is it possible to provide the authoritative (reliable) results of analytical measurements.

In Figure 2.1, a schematic presentation of the elements of quality assurance/quality control system used for obtaining reliable analytical results is shown [7].

The elements of the quality system are interdependent. To assure measuring traceability, it is indispensable to use both the certified reference materials and the analytical procedures subject to prior validation.

During the validation of an analytical procedure, it is necessary to:

- use certified reference materials – determine the accuracy,
- participate in the interlaboratory comparisons – determine the traceability, reproducibility (ruggedness),
- estimate uncertainty – which enables the control of the entire analytical procedure.

Interlaboratory comparisons involve both reference materials and validated analytical procedures. On the other hand, this type of research serves to determine certified values for the manufactured reference materials.

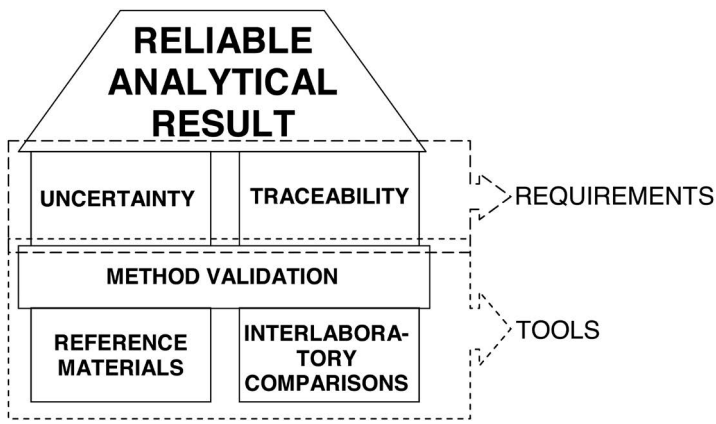


Figure 2.1 Position and role of the quality assurance/quality control (QA/QC) system elements for obtaining a reliable analytical result.

In the production of reference materials, validated analytical procedures are applied during the determination of homogeneity and stability of materials. Reference material is also characterized by the uncertainty value.

Estimation of measurement uncertainty, as noted earlier, is indispensable in the production of reference materials.

Although the uncertainty is not one of the validation parameters, it is obvious that the determination of uncertainty increases the reliability of the obtained results. It is because during the design of the so-called "uncertainty budget", it is requisite to determine the influence of all the possible parameters of an analytical procedure on the value of the combined uncertainty. This, in turn, compels the precise and very attentive "tracking" of the entire analytical procedure, those enabling the control of the procedure.

Interrelations among the particular *QA/QC* system components are presented in Figure 2.2.

Each element of the quality control system concerning the results of analytical measurements must be applied by any laboratory that

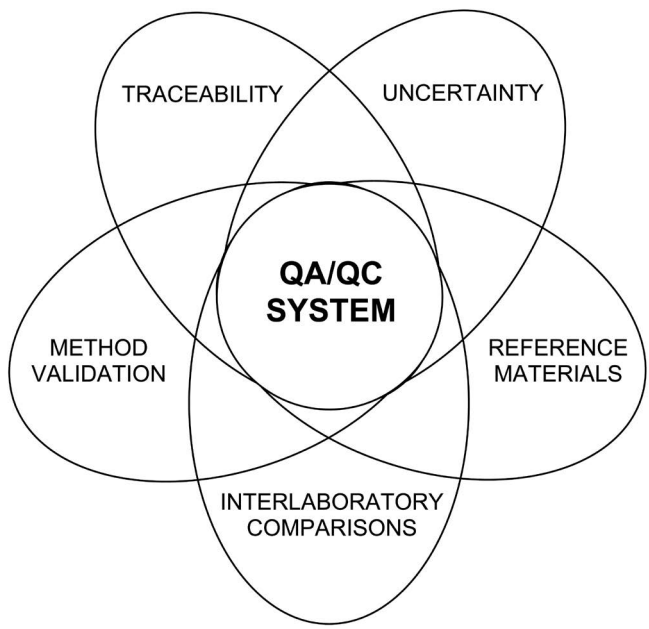


Figure 2.2 Components of the QA/QC system of an analytical process, showing interrelationships between components.

wishes to obtain reliable results. Each of these elements, in order to be applicable, must be defined in a way that is intelligible for the user. It must also be clearly and intelligibly presented, along with the determination and the control of the elements of the quality control. This can be achieved by:

- defining the basic notions of the quality system,
- determining the simple and intelligible procedures used when using individual elements of the quality system,
- providing clear and transparent dependencies (in which elements of metrology and mathematical statistics are used) enabling the "numerical" or "parametric" determination of each characteristic, and the determination of quality of the control system elements,
- helping users to derive inferences on the quality system, based on determined values for each of its elements.

Every analyst should be aware that the basic and requisite parameters characterizing an analytical result are traceability and uncertainty. These two parameters are the basic requirements for a reliable measurement result. A schematic representation of this concept is shown in Figure 2.3.

The necessity of presenting the result together with these two basic parameters must be remembered by every "producer" of an analytical result.

A requisite condition of assuring the appropriate quality of analytical results is the verification of the reliability of the used gauges and

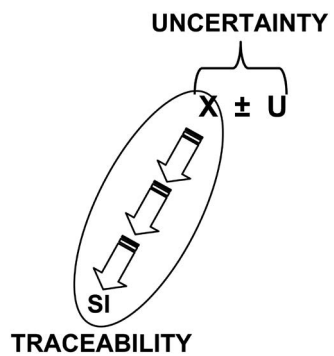


Figure 2.3 Necessary parameters for a reliable analytical result.

checking of the range of application and calibration of the analytical procedures. Accordingly, analytical procedures usually involve two operations associated with calibration:

- periodic reliability test of indications of the instruments used by means of standard mixtures; a special case of such mixtures are "zero" mixtures used for:
  - testing the zero position on the measuring scale of the instrument,
  - diluteness of standard mixtures, containing strictly defined concentrations of analytes,
- testing the reliability of the whole plot of the analytical conduct.

Realization of this operation can be achieved in two ways:

- by addition of a standard to the analyzed sample,
- as a result of applying reference material samples.

Chemical analysis of any material can be described as a chain of decisions, actions and procedures [8]. As in the case of any chain, also in a chemical analysis, the power of the entire chain also depends on the power of its weakest link. In general, the weakest links in the analytical process are not the elements acknowledged as components of chemical analysis (e.g. chromatographic extraction of mixtures or spectrometric detection), but rather the stages that take place outside the analytical laboratory, such as:

- selection of materials to be sampled,
- preparation of the sampling strategy,
- selection and use of techniques and devices necessary in sampling, and also their transport, maintenance and storage.

If a given analytical laboratory is not responsible for the sampling stage, the quality management system does not take into account these weak steps of the analytical process. Moreover, if stages of sample preparation (extraction, purifying extracts) have not been carried out properly, then even the most modern analytical instruments and complex computer techniques cannot improve the situation. Such analytical results have no value and instead of being a source of information can cause serious misinformation. Hence, QA/QC of the analytical

results should involve all stages of the analytical process. This process must be an integral, where the applicability test for the analytical method (validation) is only one stage, albeit an important one.

2.4 Conclusions

For a laboratory to be able to deliver reliable and repeatable results, it is necessary to perform systematical calibration of analytical instruments and subject all analytical procedures to validation. This notion means the determination of the methodology characteristics, covering the previous notion of "method applicability range" (selectivity, accuracy, precision, repeatability, limit of detection, range, linearity, etc.). For the purpose of quality control in a laboratory work, reference material samples are subject to the same processing and determinations as real samples. Comparison of the obtained result with the real analyte concentration in the reference material sample may give conclusions concerning the reliability of analytical works conducted in a given laboratory [9].

References

1. Konieczka P., and Namieśnik J. (eds.), Kontrola i zapewnienie jakości wyników pomiarów analitycznych, WNT, Warsaw 2017 (*in Polish*).
2. Maj-Żurowska M., Pyrzyńska K., Wagner B., and Palińska-Saadi A., *Współczesna chemia analityczna*, Second edition, PWN, PZWL Warsaw, 2022 (*in Polish*).
3. Paneva V.I., and Ponomareva O.B., Quality assurance in analytical measurements, *Accred. Qual. Assur.*, 4, 177–184, 1999.
4. Richter W., How to achieve international comparability for chemical measurements, *Accred. Qual. Assur.*, 5, 418–422, 2000.
5. Hembeck H.-W., GLP and other quality assurance systems – a comparison, *Accred. Qual. Assur.*, 7, 266–268, 2002.
6. Dobecki M. (ed), Zapewnienie jakości analiz chemicznych, Instytut Medycyny Pracy im. Prof. J. Nofera, Łódź 2004 (*in Polish*).
7. Konieczka P., The role of and place of method validation in the quality assurance and quality control (QA/QC) system, *Crit. Rev. Anal. Chem.*, 37, 173–190, 2007.
8. Valcárcel M., and Rios A., Analytical chemistry and quality, *Trends Anal. Chem.*, 13, 17–23, 1994.
9. Kellner R., Mermet J.-M., Otto M., Valcárcel M., and Widmer H.M. (eds.), *Analytical Chemistry: A Modern Approach to Analytical Science*, Second edition, Weinheim: Wiley-VCH, 2004.

3
Internal Quality Control

3.1 Definitions

Internal quality control (IQC) is the set of procedures undertaken by laboratory staff for the continuous monitoring of operation and the results of measurements in order to decide whether results are reliable enough to be released.

3.2 Introduction

IQC is extremely important to ensure that the data released from the lab are "fit for purpose". Quality control methods enable to the monitor the quality of the data produced by the laboratory on a run-by-run basis [1–3].

The laboratory should run the control samples together with the routine samples. The control values are plotted in a control chart. In this way, it is possible to demonstrate that the measurement procedure executes within the given limits. If the obtained value is outside the control limits, no analytical results are reported and corrective actions must be taken to identify the error sources and remove such errors.

If the laboratory is accredited, the standard ISO/IEC 17025 requires that the laboratory assesses the needs of the user, before any analysis. Each laboratory should define its quality requirements [4].

IQC takes place within the analytical series or runs. The main purpose of IQC is to answer the question: does my method consistently fit for purpose?

Once the laboratory has implemented a method in their daily work, is performing adequate QC, has taken appropriate corrective and/or preventive actions and its staff has acquired sufficient expertise, it may consider including this method in its scope of accreditation [5–8].

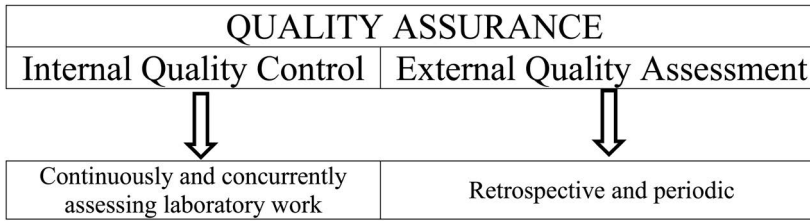


Figure 3.1 Internal quality control and external quality assessment as a part of QA/QC system.

3.3 Quality Control in the Laboratory

Quality in the laboratory can be control on two levels: internally and externally. both are characterized schematically in Figure 3.1.

The main objectives of QC in the laboratory are as follows [9]:

- to help lab staff to establish, manage and monitor a testing process to assure the analytical quality of the test results,
- to determine problems and solve them,
- to develop uniform standard of laboratory,
- to increase lab staff and client confidence,
- to create good database for decision makers,
- to reduce cost.

On the other hand, the main goals of QC are to:

- detect significant errors rapidly,
- report out good results in a timely manner,
- be cost-effective and simple to use,
- identify the sources of the errors when they occur.

There are lots of analytical factors which can influence quality. We can include some of them below [10]:

- proficiency of the personnel – education, training, competence, commitment, adequate number, supervision and motivation,
- reagents stability, integrity and efficiency – stable, efficient, desired quality, continuously available, checked (e.g. purity),
- equipment reliability – meet technical needs, compatible, user and maintenance friendly, cost-effective, validated (known value of metrological parameters), adequate space, storage

and segregation for incompatible activities, controlled and monitored environmental conditions, suitable location, "fit for purpose" – validation, calibration – documented program, maintenance, records,
- selectivity and sensitivity of selected procedure – validated, Standard Operating Procedure including every step of analytical procedure,
- use of appropriate controls,
- use of appropriate recording and documentation including all the written policies, plans, procedures, instructions and records, quality control procedures and recorded test results involved in providing a service or the manufacture of a product.

The IQC has to be planned to avoid unnecessary activities, on the one hand and not taking account all the parameters, on the other. The planning of quality control has to include [9]:

- checking the appropriate concentrations and types of control samples according to the scope of the laboratory's method,
- definition of purpose of each control: whole method, part of method (e.g. control of calibration drift),
- taking into account control at the beginning and end of each series,
- intermediate checking of the stability of the measurement process and stability of samples,
- selection what goes into database for the generation of update on general quality of analyses (when plotting results).

Data obtained during IQC can be used (even should be used) as an element of uncertainty budget for the procedure. It is mainly used as an uncertainty arising from precision, as is presented schematically on Figure 3.2.

During IQC, several tools can be used:

- standards,
- standard solutions,
- blank samples,
- appropriate CRM,
- fortified samples,
- statistical treatment – mainly control charts.

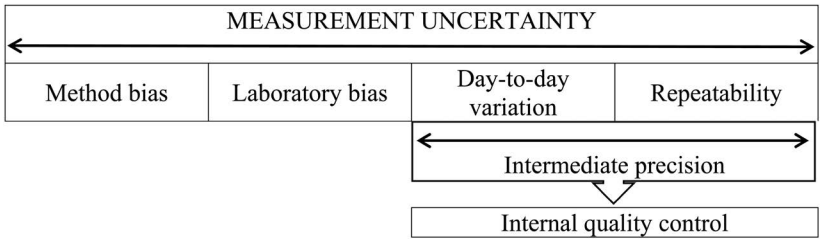


Figure 3.2 The place of IQC in uncertainty budget preparation.

3.4 Control Charts

3.4.1 Shewhart Charts

Control charts are used to test the stability of research results conducted in a given laboratory. In practice, the most frequently used charts are Shewhart charts. This method of monitoring and regulating processes is a graphic procedure minimizing the number of necessary numerical operations and allowing systematic monitoring of the course of the process being subjected to control. It enables fast and simple detection of abnormalities in the configuration of the marked points, and thus fast correction and confirmation of the reliability of the research [11–14].

The main role is played by an appropriate control chart, usually a graph with control limits depicted. On such a graph, the values of a certain statistics measurement are registered. The measurement is obtained from a series of measurement results obtained at approximately regular intervals, expressed either in time (e.g. every hour) or quantity (e.g. every batch).

The two types of variability in the charts are as follows:

- variability due to random change,
- real variability of the parameter in the process.

3.4.2 Shewhart Chart Preparation

Preparation of a chart depicting mean and standard deviation ($x_m - SD$) will be described as an example; charts are prepared separately for each procedure [12–16].

The course of action in preparing the control chart is as follows:

- Conduct 10–20 measurements for a standard sample.
- Calculate the mean x_m and the standard deviation *SD*; both values should be determined for the unbiased series, that is, after the initial rejection of outliers.
- Test the hypothesis about a statistically insignificant difference between the obtained mean and the expected value using the Student's *t* test (Section 1.8.9).
- If the hypothesis is not rejected, start preparation of the first chart:
  - Mark the consecutive numbers of result determinations on the *x*-axis of the graph, and the values of the observed characteristics (the mean) on the *y*-axis.
  - Mark a central line CL on the graph corresponding to the reference values of the presented characteristic, and two statistically determined control limits, one line on either side of the central line; the upper and lower control limits (UAL and LAL, respectively), or in other words the upper and lower warning limits. Both the upper and lower limits on the chart are found within ± 3×SD from the central line, where SD is the standard deviation of the investigated characteristics. Limits of ± 3×SD (so-called action limits) show that approximately 99.7% of the values fall in the area bounded by the control lines, provided that the process is statistically ordered. The possibility of transgressing the control limits as a result of random incident is insignificantly small; hence, when a point appears outside the control limits ± 3×SD, it is recommended that action be taken on the chart. Limits of ± 2×SD are also marked; however, the occurrence of any value from a sample falling outside these limits is simply warning about a possible transgression of the control limits; therefore, the limits of ± 2×SD are called warning limits (UWL and LWL).
  - Mark the obtained measurement results for 20 consecutive samples, but the results obtained for control samples

should be marked parallel to the received results for the investigated samples:

- If a determination result is located within the warning limits, it is considered satisfactory.
- The occurrence of results between the warning limits and action limits is also acceptable; however, not more often than two results per 20 determinations.
- If a result for a test sample is found outside the action limits, or seven consecutive results create a trend (decreasing or increasing), calibration should be carried out again.
- There exist three other signs indicating the occurrence of a problem in the analyzed arrangement, namely:
  - Three consecutive measurement points occurring outside the warning limits, but within the action limits.
  - Two subsequent measurement points being outside warning limits, but in the interval determined by the action limits, on the same side of the mean value.
  - Ten consecutive measurement points being found on the same side of the mean value.

3.4.3 Shewhart Chart Analysis

For each new chart, it is necessary to compare the mean obtained for test samples with the expected value. When the difference between these values is statistically significant, the results from this series (chart) should be rejected. Otherwise, one should compare the standard deviations obtained for the investigated chart and those obtained for a previous chart using the Snedecor's F test (Section 1.8.5), albeit the comparison should always involve two last charts. If the standard deviations do not differ in a statistically significant manner, the standard deviation is calculated for the next chart as the square root of arithmetic mean of the variances (V) values for the compared charts.

Compare the mean values obtained for the investigated chart and those obtained for a previous chart using the Student's t test (Section 1.8.9). If the means do not differ in a statistically significant manner, a new mean is calculated for the next chart as the arithmetic

mean for the compared chart, and a new chart is prepared for the newly calculated values of x_m and *SD*.

If the standard deviations differ in a statistically significant manner, a new chart should be prepared for the values of the penultimate chart.

If the mean values differed only in a statistically significant manner, a new chart should be prepared for parameters identical to those in the compared chart.

If the process is statistically regulated, then a control chart is the method used for continuously testing the statistical null hypothesis, testing whether the process is not changing and remains statistically regulated. If a value marked on the chart falls outside any of the control limits or the series of values reflects unusual configurations, the process is not statistically regulated. In this situation, one should detect the cause, and the process may then be halted or corrected. Once the cause has been located and eliminated, the process may be resumed and continued.

Example 3.1

Problem: Draw a *Shewhart* chart for the 20 given measurement results obtained for the test samples. Mark the central line, and the warning and action lines.

Data: series results:

	DATA		DATA
1	4.21	**11**	4.12
2	4.23	**12**	4.22
3	4.30	**13**	4.23
4	4.32	**14**	4.36
5	4.11	**15**	4.10
6	4.04	**16**	4.04
7	4.27	**17**	4.14
8	4.20	**18**	4.17
9	4.07	**19**	4.34
10	4.32	**20**	4.22

SOLUTION:

Mean	4.20
SD	0.10

Before calculating limit values, it is necessary to check if there are any outliers in the series. Equation 1.20 (Chapter 1) has been used, as the number of results is >10. For $\alpha = 0.05$ $k_\alpha = 1.65$.

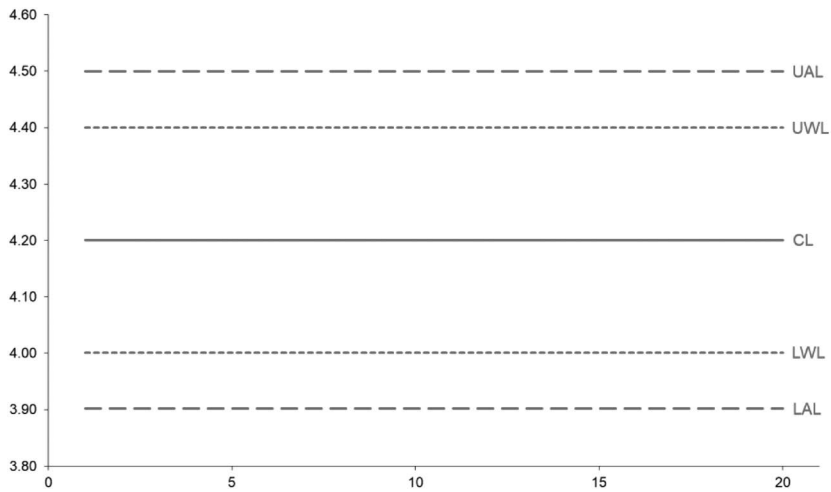
Calculated interval is equal 4.036–4.365.

All results are within the interval so there are no outliers in the series.

Calculated limits values:

UAL	4.50
UWL	4.40
LWL	4.00
LAL	3.90

Graph:



Excel file: exampl_3_1.xls

Example 3.2

Problem: On the chart in the previous example mark the following data.

Data: series results:

	DATA	
1	4.44	!
2	4.35	OK
3	4.12	OK
4	4.32	OK
5	4.18	OK
6	4.08	OK
7	4.34	OK
8	4.41	OK
9	4.23	OK
10	4.01	OK
11	4.11	OK
12	4.33	OK
13	4.20	OK
14	4.15	OK
15	4.17	OK
16	4.32	OK
17	4.00	OK
18	4.12	OK
19	4.11	OK
20	4.11	OK

SOLUTION:

$\text{Mean}_{\text{series2}}$	4.21
$\text{SD}_{\text{series2}}$	0.130

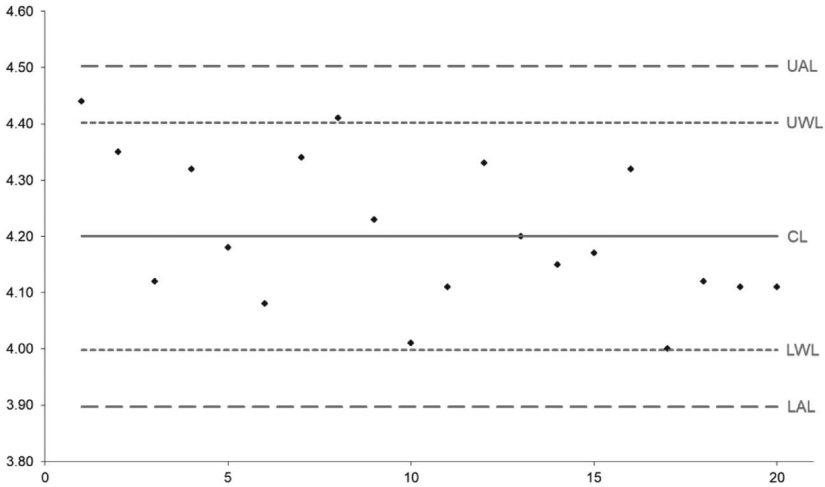
Before calculating limit values, it is necessary to check if there are any outliers in the series. Equation 1.20 (Chapter 1) has been used, as the number of results is >10. For $\alpha = 0.05$ $k_\alpha = 1.65$.

Calculated interval is equal 3.990–4.420.

The first result in the series is an outlier, so it has to be removed from the series, and new values of mean and SD have to be calculated.

Mean_2	4.19
SD_2	0.121

Graph:



Excel file: exampl_3_1.xls

Example 3.3

Problem: Draw a new chart based on the data from the previous example.

SOLUTION: Value 1 has been removed from the set of data. The remaining values were used to calculate the mean and the standard deviation.

The variances were compared using the Snedecor's F test, and then (with variances not differing in a statistically significant way), and the mean were compared using the Student's t test.

	SERIES 1	SERIES 2
No. of results – n	20	19
Standard deviation – SD	0.101	0.121
Mean	4.20	4.19
F	1.472	
$F_{crit(0.05,\ 18,\ 19)}$	2.182	
$F < F_{crit}$		
t	0.222	
$t_{crit(0.05,\ 37)}$	2.026	
$t < t_{crit}$		

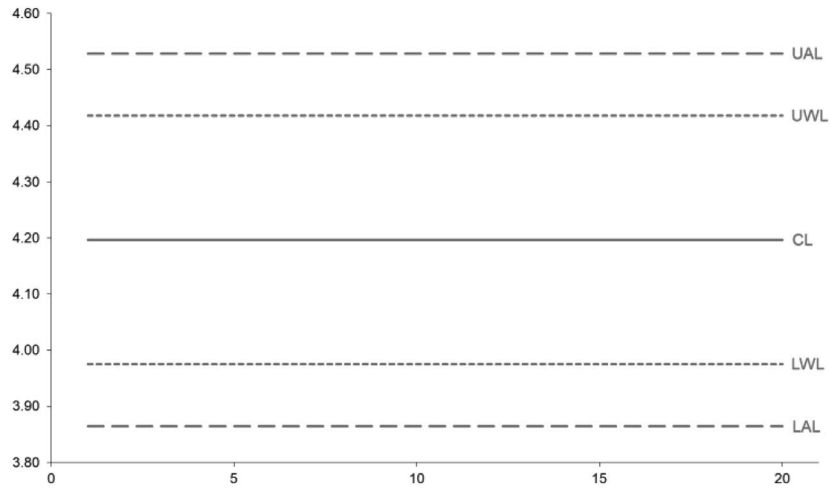
F-TEST TWO-SAMPLE FOR VARIANCES		
	VARIABLE 1	VARIABLE 2
Mean	4.19	4.20
Variance	0.0146	0.00993
Observations	19	20
df	18	19
F	1.472	
P(F ≤ f) one-tail	0.205	
F Critical one-tail	2.182	

T-TEST: TWO-SAMPLE ASSUMING EQUAL VARIANCES		
	VARIABLE 1	VARIABLE 2
Mean	4.20	4.19
Variance	0.00993	0.0146
Observations	20	19
Pooled variance	0.0122	
Hypothesized mean difference	0	
df	37	
t Stat	0.222	
P(T ≤ t) two-tail	0.825	
t Critical two-tail	2.026	

For the new chart, the values have been calculated as the means of the two previous charts.

Mean	4.20
SD	0.111
UAL	4.53
UWL	4.42
LWL	3.97
LAL	3.86

Graph:



Excel file: exampl_3_1.xls

There are 10 out-of-control situations (no possible from the probability point of view). First four are called the Western Electric Rules [12]:

1. One or more points plot outside the control limits (three-sigma limits).
2. Two out of the three consecutive points outside the two-sigma warning limits but still inside the control limits.
3. Four of five consecutive points beyond the one-sigma limits.
4. A run of eight consecutive points on one side of the center.
5. Six points in a row steadily increasing or decreasing.
6. Fifteen points in a row in zone one-sigma limits (both above and below the central line).
7. Fourteen points in a row an alternating up and down.
8. Eight points in a row in both sides of the central line with none in zone one-sigma limits.
9. An unusual or non-random pattern in the data.
10. One or more points near a warning or control limit.

For each new chart, it is necessary to compare the mean obtained for test samples with the expected value. When the difference between these values is statistically significant, the results from this series (chart) should be rejected. Otherwise, one should compare the standard deviations obtained for the investigated chart and those obtained

for a previous chart using the Snedecor's F test, albeit the comparison should always involve two last charts

$(n-1)$ and (n)

where:

$(n-1)$ – parameters for the charts,

(n) – parameters calculated from the set of data.

If the standard deviations do not differ in a statistically significant manner, the standard deviation is calculated for the next chart as:

$$SD_{n+1} = \sqrt{\frac{SD_{n-1}^2 + SD_n^2}{2}} \tag{3.1}$$

The mean values are compared using the Student's t test.

- If the means do not differ in a statistically significant manner, a new mean is calculated for the next chart as the arithmetic mean for the compared chart, and a new chart is prepared for the newly calculated values of x_m and SD.

SD and x_m is calculated based on charts $(n-1)$ and (n)

- If the standard deviations differ in a statistically significant manner, a new chart should be prepared for the values of the preultimate chart.

SD and x_m is calculated based on chart $(n-1)$

- If the mean values differed only in a statistically significant manner, a new chart should be prepared for parameters identical to those in the compared chart.

SD and x_m is calculated based on chart (n)

3.4.4 Types of Control Charts

Depending on control sample used, parameter, what to be controlled, and type of measurement, there are different types of control charts that can be used [12, 17–19]:

- **X-chart** – it is an original Shewhart chart with single values, used mainly for precision check. It can be used for trueness control but then synthetic samples with known content or RM/CRM samples may be analyzed, can be used for calibration checking (slope, intercept stability) too.
- **Blank value chart** – it is a special form of the X-chart, which can be used for the control contamination of reagents, state

(stability, selectivity) of the analytical system and contamination sources; the conclusions are made based on direct measurements of signals, not calculated values.

- **Recovery chart** – applied for controlling an influence of the sample matrix for recovery, it is calculated as:

$$\%\mathrm{R} = \left(\frac{x_{\text{spiked}} - x_{\text{unspiked}}}{\Delta x_{\text{expected}}} \right) [\%] \tag{3.2}$$

the target value around 100%.

- **Range chart (R-chart)** – the calculated parameter is an absolute difference between the highest and lowest value of multiple analyses. It can be applied for different analyte contents – then relative value can be used. This control chart has only upper limits.

Example 3.4

Problem: For given series of data calculate R-chart parameters. Make calculations for range and relative range as well.

Data: series results:

NO.	DATE	RESULT 1	RESULT 2
1	17.12.09	760	751
2	19.02.10	596	604
3	30.03.10	703	693
4	18.08.10	4706	4718
5	30.09.11	36	36.8
6	20.01.12	37.7	37.1
7	27.01.12	4205	4192
8	10.02.12	924	930
9	15.02.12	7826	7859
10	24.02.12	478	490
11	27.02.12	836	820
12	16.03.12	32	31.5
13	30.03.12	793	803
14	27.04.12	687	675
15	12.06.12	6717	6693
16	13.06.12	32.7	33.4
17	14.06.12	17.5	17.9
18	20.07.12	45	46.1
19	17.08.12	28.5	28.3
20	22.08.12	6887	6850

SOLUTION:

Range calculated for all results as $|result\ 1 - result\ 2|$

NO	R	CONCLUSION
1	9.0	+
2	8.0	+
3	10.0	+
4	12.0	+
5	0.8	+
6	0.6	+
7	13.0	+
8	6.0	+
9	33.0	+
10	12.0	+
11	16.0	+
12	0.5	+
13	10.0	+
14	12.0	+
15	24.0	+
16	0.7	+
17	0.4	+
18	1.1	+
19	0.2	+
20	37.0	-

Calculated limits values:

D4	3.267
UCL	33.7
CL	10.3

where:

CL – average value of range.

$$UCL = D4 \cdot CL$$

Conclusion: Based on limits values calculated for range, the results in row 20 are out of the UCL.

Calculation of relative range as: range/average

NO	R_{REL}, %	CONCLUSION
1	**1.2**	+
2	**1.3**	+
3	**1.4**	+
4	**0.3**	+
5	**2.2**	+
6	**1.6**	+
7	**0.3**	+
8	**0.6**	+
9	**0.4**	+
10	**2.5**	+
11	**1.9**	+
12	**1.6**	+
13	**1.3**	+
14	**1.8**	+
15	**0.4**	+
16	**2.1**	+
17	**2.3**	+
18	**2.4**	+
19	**0.7**	+
20	**0.5**	+

Calculated limits values:

D4	3.267
UCL	4.4%
CL	1.3%

Conclusion: Based on limits values calculated for relative range, no value is out of UCL.

Due to different analyte contents, the correct way of calculations is the one which used relative range.

Excel file: exampl_3_2.xls

- **CUSUM chart (CUMulative SUM)** – it is a highly sophisticated control chart. The CUSUM is a sum of all differences from one target value, whose value is subtracted from every control analyses and the difference added to the sum of all previous differences. The recognition of a systematic change

in the mean value is very simple, and it is possible to determine the order of magnitude by which the mean value has changed and the point in time at which the change occurred; the main advantages of that chart are as follows [11, 14]:

- an indication at what point the process went out of control,
- the average run length is shorter,
- the number of points that have to be plotted before a change in the process mean is detected,
- the size of a change in the process mean can be estimated from the average slope,

reference value (k) could be either an assigned value (CRM, RM, spiked sample) or the value determined in the preliminary period; the standard deviation is determined in the training period, the V-mask is the base of two-sided statistical test and is defined by the parameters:

- d, expressed in abscissa units, is the distance from the vertex of the mask to the more recent entry on the chart,
- θ is the angle between the arms of the mask and the horizontal drawn through the mask vertex,

the V-mask is usually drawn on a transparent film; it is positioned on the CUSUM chart with the vertex at a distance d from the latest entry; thus, for each new entry, the mask is shifted one abscissa unit parallel to the time axis; an out-of-control situation is indicated if the CUSUM line crosses one of the arms of the V-mask. If the CUSUM line cuts the upper arm, then the mean value has decreased and vice versa; the first CUSUM value that lies outside of the mask indicates the point in time at which the out-of-control situation appeared; this information can be helpful when searching for the cause of the error; the larger the values of d and θ, the more infrequently and out-of-control situation arises.

Example 3.5

Problem: Draw a *Shewhart* chart for the 20 given measurement results obtained for the test samples. Mark the central line, and the warning and action lines.

Calculate also data for CUSUM chart and make an appropriate graph.

Data: series results:

	DATA
1	42
2	44
3	43
4	42
5	44
6	41
7	44
8	42
9	40
10	41
11	38
12	39
13	40
14	42
15	41
16	40
17	38
18	38
19	39
20	41

Target	42
SD	3

SOLUTION:

Before calculating limit values, it is necessary to check if there are any outliers in the series. An equation 1.20 (Section 1) has been used, as the number of results is >10. For $\alpha = 0.05$ $k_\alpha = 1.65$.

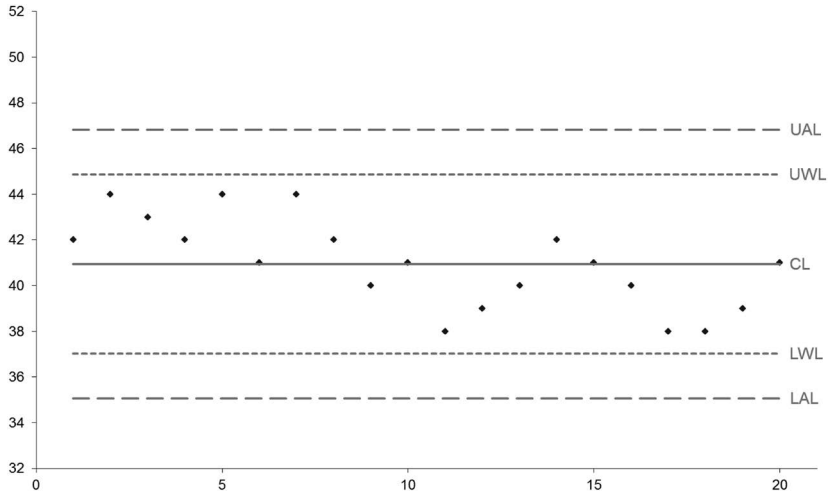
Calculated interval is equal 37.7 – 44.2.

All results are within the interval, so there are no outliers in the series.

Calculated limits values:

Mean	41.0
SD	2.0
UAL	46.8
UWL	44.9
LWL	37.0
LAL	35.1

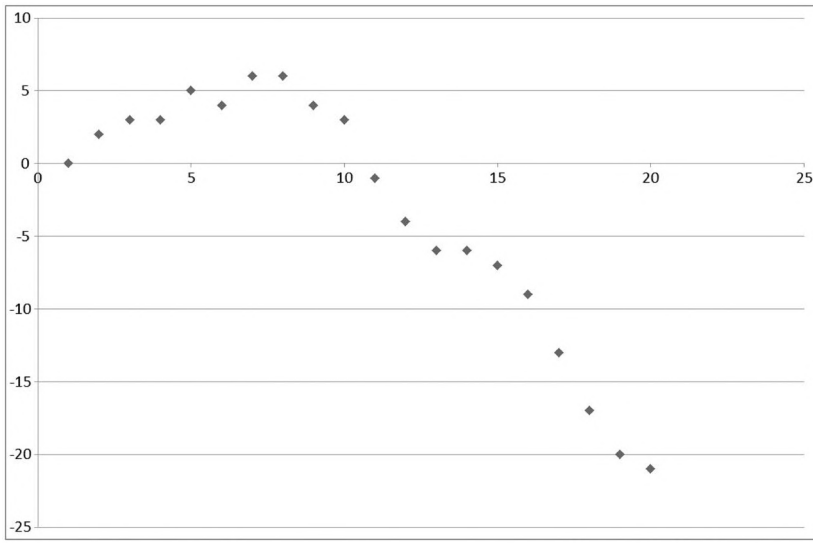
Graph:



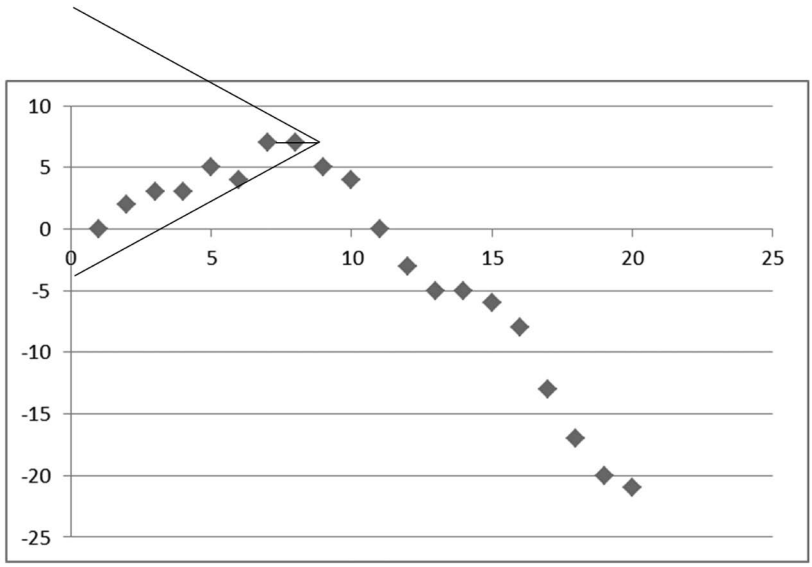
Calculations for CUSUM chart:

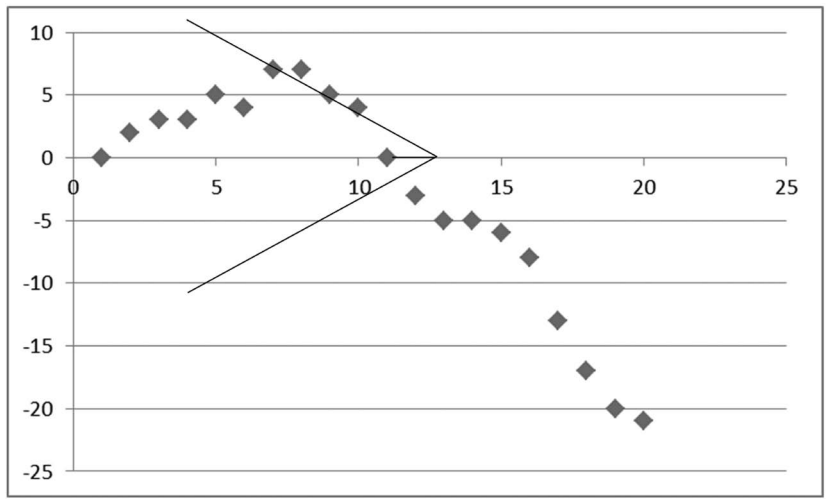
	DATA	CUSUM
1	42	0
2	44	2
3	43	3
4	42	3
5	44	5
6	41	4
7	44	6
8	42	6
9	40	4
10	41	3
11	38	−1
12	39	−4
13	40	−6
14	42	−6
15	41	−7
16	40	−9
17	38	−13
18	38	−17
19	39	−20
20	41	−21

Graph:



After putting on V-mask





This is a moment when CUSUM chart detects abnormal situation.

Excel file: exampl_3_3.xls

3.4.5 Control Samples

Appropriate control samples, used for control charts, have to fulfil below depicted requirements [11, 14]:

- be representative for matrix and analyte concentration, concentration in the region of analytically important values (limits!)
- be homogeneous
- be stable for at least several months under defined storage conditions
  - regular removal of sample aliquots for the control analyses must not lead to changes in the control sample
  - be enough available

The use of control samples must be decided taking into account a compromise/balance situation between the cost and time, and the risk to undetected analytical errors.

In order to avoid the effects of an unknown cyclicity or to detect them by applying different types of control samples, it is possible to control different parameters:

- Control samples – standards – it can be used to verify the calibration, but control sample must be completely independent from calibration solutions. The influence of sample matrix cannot be detected, and the control precision and trueness (no matrix effect) are limited.
- Control samples – blank – it can detect errors due to changes in reagents, in new batches of reagents carryover errors, and in drift of apparatus parameters; blank samples analyzed at the start and at the end allow the identification of some systematic trends.
- Control samples – real samples – it could be used for multiple analyses for range and differences charts if it is necessary to separate charts for different matrices. It can be used for rapid precision control, but it is not a way to trueness check.
- Control samples – RM, CRM – these are ideal control samples, but they are too expensive or not available for all types of analyses; in-house reference materials are a good alternative. One can be checked regularly against a CRM, and the retained sample material from interlaboratory tests can be used.

The general information about suitability of different control samples is presented in Table 3.1.

Table 3.1 Suitability of Different Types of Control Samples

SAMPLE TYPE	TRUENESS	PRECISION
CRM	Yes	Yes
QCM (RM)	Yes (CRM)	Yes
PT sample	Yes	Yes
Real sample	No	Yes
Spiked real sample	Yes (% recovery)	Yes
Blank sample	Yes (blank value)	Yes (blank value)
Synthetic sample	Yes (if representative)	Yes (if representative)
Standard solution	Yes (calibration)	Yes (calibration)

It is necessary to point that the more frequent a specific analysis is done, the more sense a control chart makes. If the analyses are always done with the same sample matrix, the sample preparation should be included. If the sample matrix varies, the control chart can be limited to the measurement only.

3.5 Conclusion

Internal quality control in chemical analytical laboratory is the continuous, critical assessment of its own analytical laboratory methods and procedures. This control includes the analytical process, starting with the sample entering the laboratory and finishing with an analytical report. The most important tool in quality control is the use of control charts. The basic recommendation is to analyze control samples in parallel with the analysis of routine samples.

The results of control can be used in several ways – the analyst will have a very important tool in its daily work. The client may get the impression of laboratory quality, and laboratory results can be used in the estimation of the uncertainty of measurement. IQC is a part of the quality system and has to be formally reviewed at regular intervals. That control could be treated as a continuous process during operational lifetime of an analytical method, while validation is a periodic one. Schematically, it is presented in Figure 3.3.

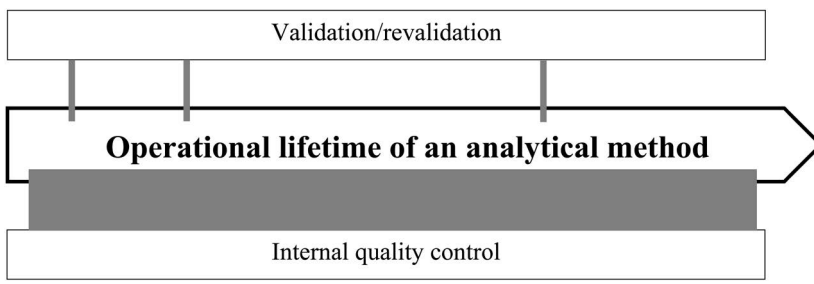


Figure 3.3 Frequency of internal quality control and method validation during operational lifetime of an analytical method.

References

1. Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., De Jong, S., Lewi, P.J., and Smeyers-Verbeke, J., *Handbook of Chemometrics and Qualimetrics, Part A*, Elsevier, 1997.
2. Mullins E., *Statistics for the Quality Control Chemistry Laboratory*, The Royal Society of Chemistry, 2003.
3. Wenclawiak, B.W., Koch, M., and Hadjicostas E. (eds.), *Quality Assurance in Analytical Chemistry, Training and Teaching*, Second edition, Springer, 2014.
4. Funk, W., Dammann, V., and Donnevert G., *Quality Assurance in Analytical Chemistry: Applications in Environmental, Food, and Materials Analysis, Biotechnology, and Medical Engineering*, Second edition, Wiley-VCH, 2008.
5. Thompson, M., and Lowthian, P.J., Effectiveness of analytical quality control is related to the subsequent performance of laboratories in proficiency tests, *Analyst*, 118, 1495–1500, 1993.
6. Royal Society of Chemistry, and Analytical Methods Committee, Internal quality control of analytical data, *Analyst*, 120, 29–34, 1995.
7. Thompson, M., and Wood, R., Harmonized guidelines for internal quality control in analytical chemistry laboratories, *Pure Appl. Chem.*, 67, 649–666, 1995.
8. Gardner, M.J., Quality control techniques for chemical analysis: some current shortcomings and possible future developments, *Accred. Qual. Assur.*, 12, 653–657, 2007.
9. Nordtest Technical Report. Internal Quality Control. *Handbook for Chemical Laboratories*, 5.1 edition, available at: http://www.nordtest.info/wp/wp-content/uploads/2018/04/NT_TR_569_ed5_1_Internal_Quality_Control_English.pdf (access date 26.06.2024).
10. Thomson, M. (eds.), Internal quality control in routine analysis. AMC technical briefs No 46. RSC 2010, available at: http://www.rsc.org/images/internal-quality-control-routine-analysis-technical-brief-46_tcm18-214818.pdf (access date 26.06.2024).
11. Mullins, E., Introduction to control charts in the analytical laboratory. Tutorial review, *Analyst*, 119, 369–375, 1994.
12. Howarth, R.J., Quality control charting for the analytical laboratory. Part 1. Univariate methods. A review, *Analyst*, 120, 1851–1873, 1995.
13. Mestek, O., Pavlík, J., and Suchánek, M., Robustness testing of control charts employed by analytical laboratories, *Accred. Qual. Assur.*, 2, 238–242, 1997.
14. Mullins, E., Getting more from your laboratory control charts. Tutorial review, *Analyst*, 124, 433–442, 1999.
15. ISO 7870-1:2019. Control charts. Part 1: General guidelines.
16. ISO 7870-2:2023. Control charts. Part 2: Shewhart control charts.
17. ISO 7870-3:2020. Control charts. Part 3: Acceptance control charts
18. ISO 7870-4:2021. Control charts. Part 4: Cumulative sum charts.
19. ISO 7870-5:2014. Control charts. Part 5: Specialized control charts.

4

Traceability

4.1 Definitions [1]

Measurand – quantity intended to be measured.

Measurement standard (etalon) – realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference.

International (measurement) standard – measurement standard recognized by signatories to an international agreement and intended to serve worldwide.

National (measurement) standard – measurement standard recognized by the national authority to serve in a state or economy as the basis for assigning quantity values to other measurement standards for the kind of quantity concerned.

Primary standard – measurement standard established using a primary reference measurement procedure, or created as an artifact, chosen by convention.

Secondary standard – measurement standard established through calibration with respect to a primary measurement standard for a quantity of the same type.

Reference standard – measurement standard designated for the calibration of other measurement standards for quantities of a given type in a given organization or at a given location.

Working standard – measurement standard that is used routinely to calibrate or verify measuring instruments or measuring systems.

Traveling standard – measurement standard, sometimes of special construction, intended for transport between different locations.

Traceability – property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

4.2 Introduction

Comparison of measurement results is sensible only when they are expressed in the same units or on the same scale. The problem of traceability appeared with the first measurements carried out by man. However, the notion of traceability itself was formulated much later, in association with the development of metrological infrastructure, initially in reference to measurements of physical properties, but later with relation to chemical measurements [2].

In the ISO 9000:2015: "Quality management systems — Fundamentals and vocabulary" [3], traceability is defined as "the ability to verify the history, location, or application of an item by means of recorded identification".

In International Vocabulary of Basic General Terms in Metrology – (VIM) [1], traceability is defined not only as a property of a measurement result, but also as a property of a reference standard. In a general meaning, it can be described as a continuous and logical process which discourages weak or missing activity at any step of an analytic process, which could burden or lower the effectiveness of the entire process.

Every day throughout the world, millions of chemical analyses are carried out, and each of them has its own requirements concerning the quality of an obtained result [4]. The obtained measurement results should be traceable to respective international standards [5, 6]. For example, for mass determination, a balance should be used which is calibrated regularly via weights with a calibration certificate that describes a reference to higher-order standard weights. These, in turn, should be calibrated against the national standards related to the international prototype kilogram. Such a series of comparisons is an uninterrupted chain illustrating the very property of traceability. Knowing uncertainty values at each step of this chain of comparisons, one can qualify the uncertainty of the value measured.

The schematic presentation of traceability meaning is shown in Figure 4.1 [7], while the rationale and meaning behind it is presented in Figure 4.2.

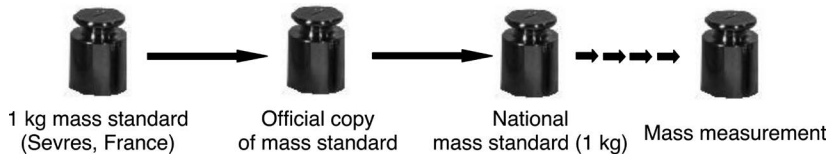


Figure 4.1 The idea of traceability – an example of mass determination [6].

The traceability could be achieved by the comparison of result value with [2]:

- SI unit,
- value represented by well-stated standard,
- value obtained by primary (absolute) method,
- value obtained by reference (excellence) laboratory,
- value obtained by group of laboratories in systematic PT scheme.

It should be noted that the value of result is traceable not to the reference material (RM) or primary method, but to the value (property) represented by or produced using its [8].

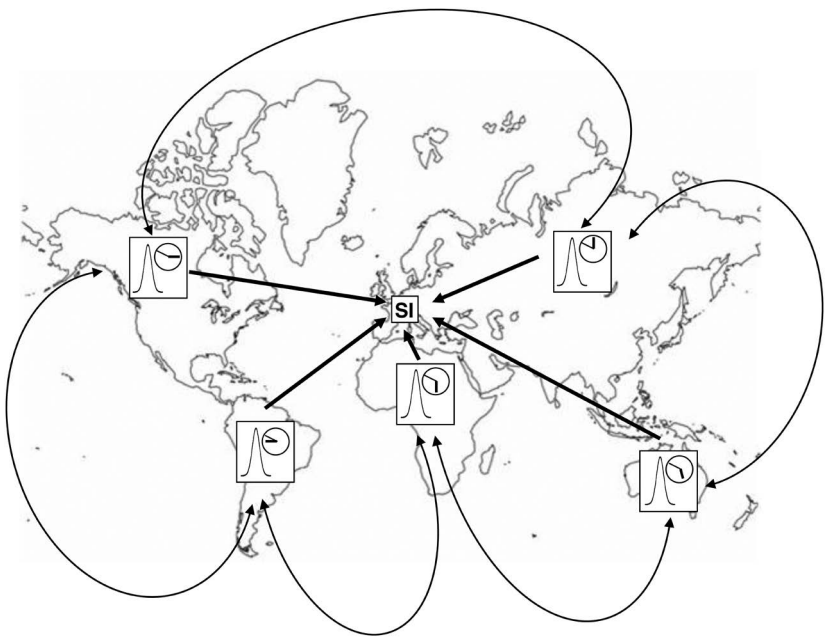


Figure 4.2 Rationale and meaning of traceability.

4.3 The Role of Traceability in Quality Assurance/ Quality Control System

The accuracy of an analytical result depends directly on the material used for calibration. For chemical measurements, if the determined substance is available as a certified reference material, then it can be treated as the last cell of an uninterrupted chain of comparisons, that is, traceability. Thus, the most important feature of reliable measurement result is its traceability in relation to the recognized standard with well-known metrological characteristics. Assurance of traceability is realized by comparing given properties to a higher-order standard. In compliance with the content of VIM [1]: "traceability is a property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties". The quoted definition of traceability underlines the elements which are especially significant in chemical measurements. Traceability is primarily a feature of a result of measurement obtained with the use of a given measurement procedure. This result must be related to a reference standard, so that it may be expressed in suitable units. Moreover, the connection should be realized by means of an uninterrupted chain of comparisons, and at every step uncertainty should be defined. A critical step of assuring traceability in chemical measurements is the applied analytical procedure because every physical or chemical operation can fracture this chain.

In compliance with the requirements of metrology, that is, the science of measurement, traceability is one of the most important elements of the quality of a result. Because results of measurements of physical and chemical properties are the basis of many decisions, respective scientific and legal metrology centers have emerged on an international scale.

In measuring physical properties, the result of a measurement depends substantially on the quality of the measuring instruments (rule, thermometer, scale) used, and in principle does not depend on the type of examined object. In measuring chemical values, apart from the scale calibration of a gauge, the result of measurement depends to a significant degree on the type of the sample and how the analytical procedure is conducted. Chemical measurements usually require sample preparation step, which means the necessity of obtaining a

representative dose of the examined material, and, for example, dissolution or mineralization of a sample, enrichment and extraction (just to mention the most important physicochemical processes).

Therefore, in chemical measurements, the notion of accuracy is difficult to define, and proving traceability is considerably more difficult. In the case of chemical measurements, there is no organized metrological system similar to physical measurements realized by a system of standardizing laboratories. In chemical measurements, calibration of instruments is not a significant source of problems. The greatest problem is assuring the traceability of the entire analytical process. As it has been noted earlier, the chain of connections with standards is always broken when a sample is physically or chemically modified in the analytical process. For this reason, an extremely important element that assures the quality of chemical measurement results is the validation of the entire measurement procedure and the estimated influence of sample components on the ultimate result of the measurement.

Traceability determination in chemical measurements is associated with many difficulties resulting from the need for sample preparation before the measurement process itself. The most important difficulties are as follows:

- identifying the object of measurement (object of determination),
- interferences,
- homogeneity of a sample (heterogeneity of composition),
- persistence of the sample,
- sample preparation,
- correctness of measurement realization,
- determination of uncertainty.

Determination of the measurand is a crucial element in the selection of an analytical procedure. In most cases, the value of the measurand depends on the applied methodology and/or on the measurement conditions. Thus, the results can only be compared in the same measurement conditions.

Interferences, that is, the influence of sample components on the analytical signal, depend on the type of determined substance (analyte) and the type of matrix of the sample. As noted earlier, when measuring physical properties, the sample type does not have a significant influence on the measurement result. In chemical measurements, a

result of determination depends on the components accompanying the analyte. For example, it is well known that a type of acid mixture used for mineralization influences the atomic absorption signal. The type of acid affects the process of atomization, and therefore the effectiveness of creating free atoms in the determined element. This effect is extremely important with consideration to the entire chemical measurement and must be taken into account when validating a given measurement procedure.

The homogeneity of a sample (heterogeneity of composition) significantly influences the determination of the representative portion of the examined material. While planning analytical conduct, an analyst must allow for the heterogeneity of a sample's composition; hence, for solid samples, an analytical sample should be sufficiently large so that grain size is not a source of heterogeneity.

The stability of a sample determines the measurement duration. In some cases, the composition of a sample can change even over several minutes, hence the necessity for exact knowledge concerning how the sample behaves over time.

The preparation of a sample is the most important element causing difficulty in maintaining traceability. Every physicochemical operation disrupts the chain of traceability, which implies a necessity for a detailed plan of action.

Correct realization of a measurement depends primarily on the efficiency of the measuring instrument used and the maintenance of suitable measurement conditions. For example, a pH measurement requires calibration of an instrument and measurements at a suitable temperature.

Determination of the uncertainty of a result is an integral part of traceability assurance. Uncertainties in reference standards comprise the uncertainty of a result obtained by a comparison with these standards.

Traceability should be shown for each parameter of a given procedure and should be carried out by calibration with suitable standards.

A procedure enabling the determination of correlations between the value of a signal (indicated by an instrument) and the concentration of the examined substance in the sample is called calibration.

It is necessary to use reference standards for which traceability may be shown and which have known uncertainty.

An important part at this step is played by reference materials, which can assure traceability to standards, that is to say, which obtain traceability, and consequently international agreement on measurements [9].

In practice, traceability for chemical measurements can be determined in two ways [10]:

- by comparing an obtained value with reference measurements,
- by referring an obtained value to reference standards, which in turn have a connection with the value obtained in reference measurements.

Reference values should come from expert laboratories with good international reputations.

For trace analysis, fulfilling the traceability requirement for a typical analytical procedure demands the use of a matrix reference materials. The traceability of measurement results depends on, among other things, the proper functioning of instruments, which can be assured by calibration using suitable calibrants. The calibration step is used for [11, 12]:

- assuring the correct performance of an instrument (instrument calibration),
- determining a clear dependence between a determined signal and a determined property (analytical calibration).

For reference materials reproducing the chemical properties, the problem of traceability assurance involves the accessibility of standards with a required level of analyte concentration, determined with a suitable accuracy (higher than the accuracy in the applied analytic methodology).

Here, it must be remembered that very frequently, analytes in samples occur in trace or ultratrace amounts, and preparing suitable reference materials poses an immense challenge.

This has an undoubtable influence on the cost of preparing reference materials.

Many reference materials may have properties that for various reasons cannot be measured in units of mass or quantity, nor determined by means of precisely defined physical and chemical measurement methods. Examples of such reference materials are biological reference materials attributed to a respective international unit by the World Health Organization, and also technological reference materials [13].

Assuring traceability, and hence assuring the reliability of measurements, is an element of analytical chemistry which is currently given considerable attention. That is why the notion of traceability and the associated notion of uncertainty are also two key problems in present-day metrology in analytical chemistry. For the purpose of obtaining a full and correct picture, traceability should be considered in four ways [14]:

- the traceability of analytical results, that is, the assurance of the obtained analytical results referred to specific reference materials by an uninterrupted chain of comparisons of uncertainties associated with suitable reference materials (certification and history of their production),
- the traceability of the applied standards, that is, the properties of standard values than can be related to reference materials by an uninterrupted chain of comparisons of uncertainties associated with suitable reference materials and supplied documentary evidence giving the history of their production (in which significant properties such as homogeneity, stability and origin must be clearly presented),
- the traceability of an instrument, that is, a detailed and up-to-date history of the instruments containing descriptions of their installation, damage, number of hours used, sample processing, and other parameters associated with the specific instrument, with special attention paid to maintenance, calibration and repairs,
- the traceability of analytical methodology (procedures), that is, the possibility of obtaining traceable results after a correct process of validating all analytical conduct.

In compliance with requirements stated in the EURACHEM/CITAC Guide [15], in order to determine the traceability of a given analytical procedure, it is necessary to:

- determine the measurand,
- select a suitable measurement procedure and record a respective model equation,
- prove (by validation) the correctness of the selected measuring conditions and the model equation,
- determine a strategy for proving traceability by selecting suitable standards and determining procedure calibration,

- determine the uncertainty of the applied measurement procedure.

As noted earlier, one of the most important tools used for the purpose of traceability assurance in chemical measurements is the use of certified reference materials, which are extremely useful for:

- estimating the accuracy of new analytical procedures,
- comparing different methods,
- comparing and testing the competence of different laboratories.

Realization of traceability in chemical measurements by means of reference materials can be carried out using pure standard substances for calibration or suitable certified reference materials. It is very important to purchase reference material from a reputable distributor, which will assure the maintenance of traceability for a given value together with a given uncertainty value. The most important criteria for the selection of reference materials are primarily the agreement of matrix and concentrations of the determined substance. Moreover, it is necessary to allow for uncertainty provided by the manufacturer and to estimate to what extent this will be important in the uncertainty budget of the applied measurement procedure.

4.4 Conclusion

The main sense of traceability is to enable comparability of measurement results – either compare results of the measurements on the same sample or compare results on different samples [12]. In theory, all measurements can be tracked back to the base seven SI units [16]. Traceability is highly connected with uncertainty, comparability, utility, reliability and validity.

Traceability and uncertainty are necessary parameters for obtaining reliable results.

References

1. International vocabulary of metrology – Basic and general concepts and associated terms (VIM), Joint Committee for Guides in Metrology, JCGM 200, 2012.
2. Valcárcel M., and Rios A., Traceability in chemical measurements for the end users, *Trends Anal. Chem.*, 18, 570–576, 1999.

3. ISO 9000:2015. Quality management systems – fundamentals and vocabulary.
4. Walsh M.C., Moving from official to traceable methods, *Trends Anal. Chem*., 18, 616–623, 1999.
5. De Bièvre P., Kaarls R., Peiser H.S., Rasberry S.D., and Reed W.P., Protocols for traceability in chemical analysis. Part II: Design and use, *Accred. Qual. Assur.*, 2, 270–274, 1997.
6. De Bièvre P., and Taylor P.D.P., Traceability to the SI of amount-of-substance measurements: from ignoring to realizing a chemist's view, *Metrologia*, 34, 67–75, 1997.
7. Thomson M., Comparability and traceability in analytical measurements and reference materials, *Analyst*, 122, 1201–1205, 1997.
8. King B., The practical realization of the traceability of chemical measurements standards, *Accred. Qual. Assur.*, 5, 429–436, 2000.
9. ISO 33405:2024: Reference materials – Approaches for characterization and assessment of homogeneity and stability.
10. Bulska E., and Taylor P., On the importance of metrology in chemistry, in: Namieśnik J., Chrzanowski W., and Żmijewska P. (eds), New Horizons and Challenges in Environmental Analysis and Monitoring, CEEAM, Gdańsk, 2003.
11. Valcárcel M., and Rios A., Is traceability an exclusive property of analytical results? An extended approach to traceability in chemical analysis, *Fresenius J. Anal. Chem*., 359, 473–475, 1997.
12. Williams A., Traceability and uncertainty – a comparison of their application in chemical and physical measurement, *Accred. Qual. Assur.*, 6, 73–75, 2001.
13. ISO Guide 30:2015, Reference materials – Selected terms and definitions.
14. Marschal A., Andrieux T., Compagon P.A., and Fabre H., Chemical metrology – QUID?, *Accred. Qual. Assur.*, 7, 42–49, 2002.
15. EURACHEM/CITAC Guide, Metrological Traceability in Chemical Measurements. A guide to achieving comparable results in chemical measurement, Second Edition 2019, available at: https://www.eurachem.org/images/stories/Guides/pdf/ECTRC_2019_EN_P1.pdf (access date 26.06.2024).
16. Buzoianu M., and Aboul-Enein H. Y., The traceability of analytical measurements, *Accred. Qual. Assur.*, 2, 11–17, 1997.

5 Uncertainty

5.1 Definitions [1–4]

Uncertainty of measurement – nonnegative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.

Definitional uncertainty – component of measurement uncertainty resulting from the finite amount of detail in the definition of a measurand.

Standard uncertainty $u_{(x_i)}$ – uncertainty of a result x_i of a measurement expressed as a standard deviation.

Combined standard uncertainty $u_{c(y)}$ – standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model; standard uncertainty of a result y of a measurement when the result is obtained from the values of many of other quantities equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with these quantities.

Uncertainty budget – statement of a measurement uncertainty, of the components of that measurement uncertainty, and of their calculation and combination.

Expanded uncertainty U – product of a combined standard measurement uncertainty and a factor larger than the number 1.

Coverage factor k – number larger than 1 by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty; a coverage factor is typically in the range of 2–3, and for an approximately 95% level of confidence, $k = 2$.

Type A evaluation (of uncertainty) – evaluation of a component of measurement uncertainty by a statistical analysis of

measured quantity values obtained under defined measurement conditions.

Type B evaluation (of uncertainty) – evaluation of a component of measurement uncertainty determined by means other than a type A evaluation of measurement uncertainty.

Relative uncertainty $u_{r(x_i)}$ – standard measurement uncertainty divided by the absolute value of the measured quantity value.

5.2 Introduction

Decisions made in many fields of science and other domains of life are based on the results of analytical studies. It is therefore obvious that their quality is increasingly important.

Uncertainty of measurement is a component of uncertainty for all individual steps of an analytical procedure [5–8]. Hence, it is necessary to determine the sources and types of uncertainty for all these steps [9–11].

The main sources of uncertainty during sample analysis while using an appropriate analytical procedure may be [12]:

- inaccurate or imprecise definition of the measurand,
- lack of representativeness at the step of collecting a sample from an examined material object,
- inappropriate methodology of determinations,
- personal deviations in reading the analog signals,
- not recognizing the influence of all the external factors on the result of an analytical measurement,
- uncertainty associated with the calibration of an applied measurement instrument,
- insufficient resolution of the applied measurement instrument,
- uncertainties associated with the applied standards and/or reference materials,
- uncertainties of parameters determined in separate measurements and which are used in calculating the final result; such as physicochemical constants,
- approximations and assumptions associated with using a given instrument, applied during measurement,
- fluctuations of the measurement instrument gauge, over the course of repeated measurements, with seemingly identical external conditions.

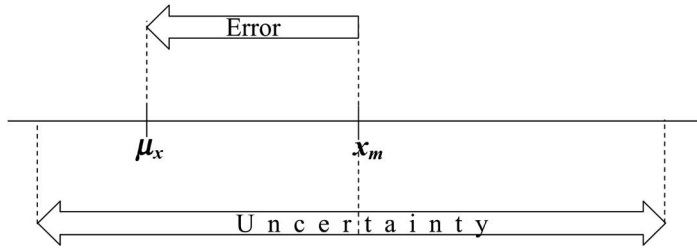


Figure 5.1 Schematic presentation of the difference between error and measurement uncertainty [12].

There is a difference between measurement error and uncertainty. The error is a difference between the determined and expected values, and uncertainty is a range into which the expected value may fall within a certain probability. So the uncertainty cannot be used to correct a measurement result.

This difference is schematically presented in Figure 5.1 [12].

5.3 Methods of Estimating of Measurement Uncertainty

There are several approaches for uncertainty estimation [13, 14]:

- *bottom-up* – based on an identification, quantification, and combination of all individual sources of uncertainty of measurement. The overall uncertainty is derived from the uncertainties of individual components. This method has high complexity and because of that it needs considerable time and effort; this approach is adapted by EURACHEM [2, 3, 15],
- *fitness-for-purpose* – based on a definition of single parameter called the fitness function, which has the form of algebraic expression, and describes the relation between uncertainty and analyte content. Calculation of uncertainty for the result of measurement is very easy and less time-consuming than a *bottom-up* approach,
- *top-down* – based on data obtained from interlaboratory studies (precision),
- *validation-based* – based on inter- or intralaboratory validation studies (precision, trueness, robustness),
- *robustness-based* – based on robustness tests from interlaboratory studies.

5.3.1 *Procedure for Estimating the Measurement Uncertainty According to Guide to the Expression of Uncertainty in Measurement*

Determining the uncertainty of a measurement increases its reliability, and in turn allows comparison of results obtained in interlaboratory studies and helps users to decide the significance of any difference between the obtained result and the reference value.

According to the Guide to the Expression of Uncertainty in Measurement [2, 3], in order to determine the uncertainty of analysis result, the following conditions must be satisfied:

1. The measurement procedure and the measurand must be defined.

   The measurand in a given measurement must be clearly defined, along with the unit in which it is expressed. The observed quantity and the searched parameter (result) must also be clearly described.
2. Modeling (usually mathematical modeling) must be applied to calculate the analysis result based on the measured parameters.

   An appropriate mathematical model ties the value of a determination result (the one to be determined) with the observed values (measurement values). The relation is described as follows:

$$y = f(x_1, x_2 \cdots x_n) \tag{5.1}$$

   where:

   y – value of a result,

   $x_1, x_2 \ldots x_n$ – measurement values.
3. Values must be assigned to all the possible parameters that could affect the final result of the analysis, and the standard uncertainty for each of them must be determined.

   Each measurand has a name, unit, value, standard uncertainty and its number of degrees of freedom. As noted before, there are two methods for calculating standard uncertainty. Type A uncertainty is equal to a standard deviation of an arithmetic mean. Type B uncertainty is strictly associated with the probability distribution described by the distribution of a variable.

For example, when a variable has a rectangular distribution, such as in the case of a standard's purity, the variable may assume (with equal probability) a value in the range $\langle -a, +a \rangle$, and the calculated standard uncertainty is $\frac{a}{\sqrt{3}}$ (where a is the midpoint of the range $\langle -a, +a \rangle$).

When a variable has a triangular distribution, which means that the value is in the range $\langle -a, +a \rangle$, but the occurrence of the mean value from the range is the most probable, the calculated standard uncertainty is $\frac{a}{\sqrt{6}}$.

Example 5.1

Problem: Calculate standard uncertainty for the concentration of magnesium in a standard solution, based on data given by producer.

Data: standard solution concentration C_{st} = 1001 ± 2 mg/dm^3

SOLUTION: Due to no additional information, we assume a uniform distribution,

$$u_{(C_{st})} = \frac{2}{\sqrt{3}}$$

$$u_{(C_{st})} = 1.2\, mg/dm^3$$

Excel file: exampl_5_1.xls

Example 5.2

Problem: Calculate the standard uncertainty for the concentration of magnesium in a standard solution, based on data given by producer. Uncertainty given by the manufacturer have been calculated for coverage factor k = 2.

Data: standard solution concentration C_{st} = 1001 ± 2 mg/dm^3

SOLUTION: Because value for k is given, standard uncertainty is calculated accordingly:

$$u_{(C_{st})} = \frac{2}{k}$$

$$u_{(C_{st})} = 1 \mathrm{mg/dm^3}$$

Excel file: exampl_5_2.xls

Example 5.3

Problem: Calculate the standard uncertainty for the determination of volume 500 cm^3 using volumetric flask, based on data given by the manufacturer.

Assume triangular distribution.

Data: volume $V_{fl} = 500 \pm 0.8\ cm^3$

SOLUTION:

$$u_{(V_{fl})} = \frac{0.8}{\sqrt{6}}$$

$$u_{(V_{fl})} = 0.32 cm^3$$

Excel file: exampl_5_3.xls

4. The applied principles of uncertainty propagation in calculating the standard uncertainty of an analytical result.

For a given mathematical model that binds the final results of analysis with measured parameters (Equation 5.1), standard uncertainty is calculated by using the principle of uncertainty propagation expressed in the following formula:

$$u_c^2(y) = \sum_{i=1}^{n} \left(\frac{\delta f}{\delta x_i} \right)^2 u_{(x_i)}^2 \tag{5.2}$$

When the value of an analytical result is the sum or difference of the measurement values

$$y = x_1 + x_2 + \cdots + x_n \tag{5.3}$$

then the value of the combined uncertainty is described by the following equation:

$$u_{c(y)} = \sqrt{u_{(x_1)}^2 + u_{(x_2)}^2 + \cdots + u_{(x_n)}^2} \tag{5.4}$$

Due to the very frequent occurrence of individual measurement values being expressed in different units, it is more convenient to apply relative uncertainties. A relative uncertainty is described by the following relation:

$$u_{r(x_i)} = \frac{u_{(x_i)}}{x_i} \tag{5.5}$$

If the value of the analytical result is a quotient/product of the measurement values,

$$y = \frac{x_1 \cdot x_2 \cdot \ldots}{x_3 \cdot \ldots} \tag{5.6}$$

then the value of the combined relative uncertainty is described by the following equation:

$$u_{r(y)} = \sqrt{u^2_{r(x_1)} + u^2_{r(x_1)} + \ldots + u^2_{r(x_n)}} \tag{5.7}$$

5. Presentations of the final result of the analysis as: *result* ± *expanded uncertainty* (after using an appropriate *k* factor).

   Uncertainty calculated according to the aforementioned equation is a combined standard uncertainty of the final determination. To calculate the value of the expanded uncertainty, the combined standard uncertainty should be multiplied by an appropriate coverage factor *k*.

   Therefore, the final result of an analysis comprises the following:

   - determination of the measured value and its unit,
   - the result with the expanded uncertainty value ($y \pm U$, along with units for y and U),
   - *k* factor value, for which the expanded uncertainty has been calculated.

Thus, a correctly presented result of an analysis should be as follows:

$$c_{NaOH} \pm U(k=2) = 0.1038 \pm 0.0017 [mol/dm^3]$$

or

$$c_{NaOH} \pm U(k=2) = 0.1038\ mol/dm^3 \pm 1.6\ [\%]$$

Example 5.4

Problem: A standard Mg^{2+} solution was prepared, with a basic diluted solution with a concentration of 1001 ± 2 mg/dm^3. With the aim of obtaining a standard solution with a concentration of around 0.5 mg/dm^3, the basic solution was diluted as follows:

We took 1 cm^3 of basic sample solution by using a pipette with a volume of 1 cm^3 and transferred it to a volumetric flask with a volume of 100 cm^3. After filling the flask to the line and mixing the solution, 5 cm^3 of solution was taken from it with the help of a pipette with a volume of 5 cm^3 and was transferred to a volumetric flask with a volume of 100 cm^3 and after being filled to the line, a standard solution was obtained with the predetermined concentration.

To establish a uniform distribution for each of the measured parameters, calculate the following:

- the value of the combined and expanded uncertainty (for k=2) for the obtained standard solution concentration, and present indication results,
- the participation percentage of each of the standard uncertainty values in the determined values of the combined uncertainty.

Data:

			UNIT
Standard solution concentration	C_{st}	1001	mg/dm^3
Pipette 1 volume	V_{p1}	1	cm^3
Flask 1 volume	V_{f1}	100	cm^3
Pipette 2 volume	V_{p2}	5	cm^3
Flask 2 volume	V_{f2}	100	cm^3
Uncertainty of single measurement	$u(C_{st})$	2	mg/dm^3
	$u(V_{p1})$	0.02	cm^3
	$u(V_{f1})$	0.2	cm^3
	$u(V_{p2})$	0.03	cm^3
	$u(V_{f2})$	0.2	cm^3
Distribution	Rectangular (R) or Triangular (T)		R

SOLUTION:

x_i	u_r	RELATIVE UNCERTAINTY CONTRIBUTION*, %
C_{st}	0.0012	0.89
V_{p1}	0.012	89.29
V_{f1}	0.0012	0.89
V_{p2}	0.0035	8.04
V_{f2}	0.0012	0.89
k	2	

*calculated as: $\frac{\left(u_r(x_i)\right)^2}{\left(u_r(c)\right)^2}[\%]$.

		UNIT
c	0.5005	mg/dm^3
$u_r(c)$	0.012	
$U(c)(k = 2)$	0.012	mg/dm^3
U %	2.4	
Result	0.501 ± 0.012	mg/dm^3

Excel file: exampl_5_4.xls

Example 5.5

Problem: A standard Mg^{2+} solution was prepared, with a basic diluted solution with a concentration of 1001 ± 2 mg/dm^3. With the aim of obtaining a standard solution with a concentration of around 0.5 mg/dm^3, the basic solution was diluted as follows:

A basic standard solution of 10 cm^3 was taken using a pipette with a volume of 10 cm^3 and was transferred to a volumetric flask with a volume of 100 cm^3. After filling the flask to the line and mixing the solution, 5 cm^3 of solution was taken from it with the help of a pipette with a volume of 5 cm^3 and was transferred to a volumetric flask with a volume of 100 cm^3. After filling the flask to the line and mixing the solution, 10 cm^3 of solution was taken from it with the help of a pipette with a volume of 10 cm^3 and was transferred to a volumetric flask with a volume of 100 cm^3 and after being filled to the line a standard solution was obtained with the predetermined concentration.

To establish a uniform distribution for each of the measured parameters, calculate the following:

- the value of the combined and expanded uncertainty (for $k = 2$) for the obtained standard solution concentration, and present indication results,
- the participation percentage of each of the standard uncertainty values in the determined values of the combined uncertainty.

Data:

			UNIT
Standard solution concentration	C_{st}	1001	mg/dm^3
Pipette 1 volume	V_{p1}	10	cm^3
Flask 1 volume	V_{f1}	100	cm^3
Pipette 2 volume	V_{p2}	5	cm^3
Flask 2 volume	V_{f2}	100	cm^3
Pipette 3 volume	V_{p2}	10	cm^3
Flask 3 volume	V_{f2}	100	cm^3
Uncertainty of single measurement	$u(C_{st})$	2	mg/dm^3
	$u(V_{p1})$	0.04	cm^3
	$u(V_{f1})$	0.2	cm^3
	$u(V_{p2})$	0.03	cm^3
	$u(V_{f2})$	0.2	cm^3
	$u(V_{p2})$	0.04	cm^3
	$u(V_{f2})$	0.2	cm^3
Distribution	Rectangular (R) or triangular (T)		R

SOLUTION:

x_i	u_r	RELATIVE UNCERTAINTY CONTRIBUTION, %
C_{st}	0.0012	4.75
V_{p1}	0.0023	19.05
V_{f1}	0.0012	4.76
V_{p2}	0.0035	42.86
V_{f2}	0.0012	4.76
V_{p3}	0.0023	19.05
V_{f3}	0.0012	4.76
k	2	

		UNIT
c	0.5005	mg/dm^3
$u_r(c)$	0.0053	
$U(c)$	0.0053	mg/dm^3
U %	1.1	
Result	0.5005 ± 0.0053	mg/dm^3

Excel file: exampl_5_5.xls

Example 5.6

Problem: A standard sample was weighed for the preparation of a standard solution. The mass measurement was carried out using an analytical scale, for which its producer gave a measurement uncertainty of 0.5 mg.

The mass was calculated as the difference of two mass measurements: gross (container with a sample – 332.55 mg) and net (container – 187.72 mg). Calculate the standard uncertainty of the mass measurement. Assume a rectangular distribution of the parameter. Assuming the value of the coverage factor to be 2, calculate the expanded uncertainty of the mass measurement. Give a correct presentation of the mass measurement result.

Data:

			UNIT
Mass (tarra)	m_{tarra}	187.72	mg
Mass (brutto)	m_{brutto}	332.55	mg
Uncertainty of single measurement	$u(m_{tarra})$	0.5	mg
	$u(m_{brutto})$	0.5	mg
Distribution	Rectangular (R) or triangular (T)		R

SOLUTION:

m_{netto}	144.83	mg
$u(m_{netto})$	0.41	mg
k	2	
$U(m_{netto})$	0.82	mg
Result	144.83 ± 0.82	mg

Excel file: exampl_5_6.xls

Example 5.7

Problem: The weighed standard sample (Example 5.6) was put into a measurement flask (250 cm^3) for which the manufacturer provided a uncertainty value equal to 0.4 cm^3. Calculate the combined standard uncertainty of the obtained standard solution concentration. Assume a rectangular distribution of the parameters. Assuming the value of the coverage factor to be 2, calculate the expanded uncertainty of the concentration. Give a correct presentation of the result.

Data:

			UNIT
Mass (tarra)	m_{tarra}	187.72	mg
Mass (brutto)	m_{brutto}	332.55	mg
Flask volume	V_{flask}	250	cm^3
Uncertainty of single measurement	$u(m_{tarra})$	0.5	mg
	$u(m_{brutto})$	0.5	mg
	$u(V_{flask})$	0.4	cm^3
Distribution	Rectangular (R) or triangular (T)		R

SOLUTION:

Concentration	0.5793	mg/cm^3
u_r(concentration)	0.0020	
k	2	
U(concentration)	0.0023	mg/cm^3
Result	0.5793 ± 0.0023	mg/cm^3

Excel file: exampl_5_7.xls

Example 5.8

Problem: The obtained standard solution (Example 5.7) was dissolved by a 1:10 ratio, sampling 1 cm^3 of the original solution using a pipette for which the manufacturer provided an uncertainty value of 0.2 cm^3, and dissolving in a measurement flask (10 cm^3), for which the manufacturer provided an uncertainty value 0.05 cm^3.

Calculate the combined standard uncertainty of the obtained standard solution concentration. Assume a rectangular distribution of parameters. Assuming the value of the coverage factor to be 2, calculate the expanded uncertainty of the concentration. Give a correct presentation of the result.

Data:

			UNIT
Mass (tarra)	m_{tarra}	187.72	mg
Mass (brutto)	m_{brutto}	332.55	mg
Flask volume	V_{flask1}	250	cm^3
Flask volume	V_{flask2}	10	cm^3
Pipette	$V_{pipette}$	1	cm^3
Uncertainty of single measurement	$u(m_{tarra})$	0.5	mg
	$u(m_{brutto})$	0.5	mg
	$u(V_{flask1})$	0.4	cm^3
	$u(V_{flask2})$	0.05	cm^3
	$u(V_{pipette})$	0.2	cm^3
Distribution	Rectangular (R) or triangular (T)		R

SOLUTION:

Concentration	0.0579	mg/cm^3
u_r(concentration)	0.12	
k	2	
U(concentration)	0.13	mg/cm^3
Result	0.058 ± 0.013	mg/cm^3

Excel file: exampl_5_8.xls

5.4 Tools Used for Uncertainty Estimation

Correct estimation of uncertainty needs an understanding of the whole analytical procedure by the analyst. The most helpful tools used for that are as follows [5, 7]:

- *flow diagram* – which is drawn on the basis of an information presented in detail in a standard operating procedure,
- *Ishikawa, or cause-and-effect, or fishbone diagram* – which shows the influence parameters (sources of uncertainty) of a whole analytical procedure [16, 17].

The flow diagram and the Ishikawa diagram for the procedure of preparation of a standard solution are presented in Figures 5.2 and 5.3, respectively.

5.5 Uncertainty and Confidence Interval

In some cases, the value of uncertainty can be estimated as a confidence interval. The basic principle of the uncertainty propagation is underlining the influence of the quantity with the highest value.

Therefore, if one of the parameters has a dominating influence over the uncertainty budget, calculation of uncertainty may be limited to the calculation based on the value of that parameter. If that dominating parameter is the repeatability of measurements, then the expanded uncertainty may be calculated according to the following relation:

$$U = k\frac{SD}{\sqrt{n}} \tag{5.8}$$

where:

SD – standard deviation,

n – number of measurements.

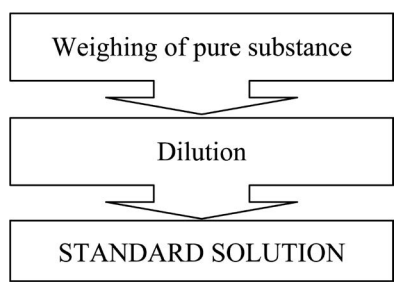


Figure 5.2 Flow diagram for the procedure of preparation of standard solution.

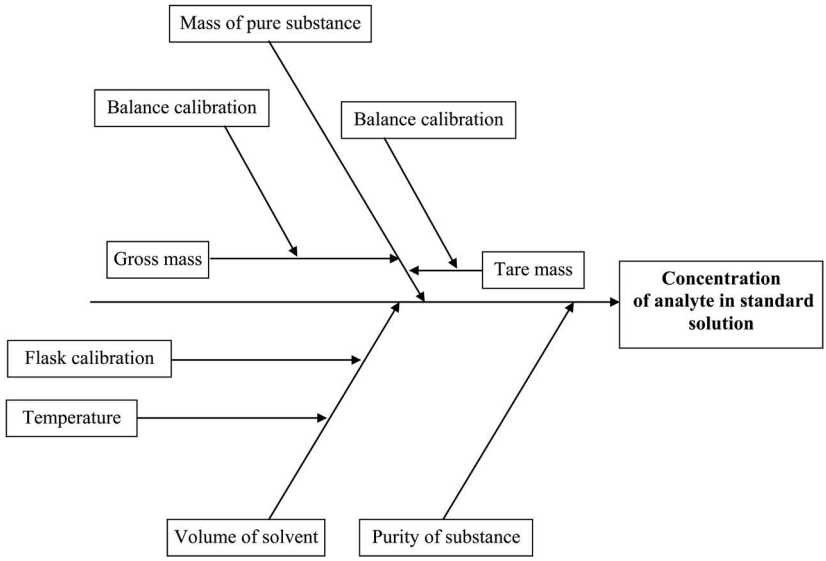


Figure 5.3 Ishikawa diagram for the procedure of preparation of standard solution.

On the other hand, the value of confidence interval could be calculated as:

$$\Delta x_{s'_r} = t(\alpha, f)\frac{SD}{\sqrt{n}} \tag{5.9}$$

For a level of significance of $\alpha = 0.05$, the coverage factor $k = 2$.

For a level of significance of $\alpha = 0.05$ and the number of degrees of freedom $f \to \infty$, the parameter $t \approx 2$ – **Table A.1**.

Given this condition, the aforementioned equations are thus consistent.

Example 5.9

Problem: The concentration of mercury was determined in water using the cold vapor atomic absorption spectrometry (CVAAS) technique. The series involved four determinations. Considering the unrepeatability as the main component of the uncertainty budget, calculate the expanded uncertainty of the determination result for $k = 2$. Provide a correct presentation of the determination result.

Data: result series, μg/dm^3:

	DATA
1	71.53
2	72.14
3	77.13
4	76.54

SOLUTION:

Mean	74.335	μg/dm^3
SD	2.91	μg/dm^3
k	2	
U	2.9	μg/dm^3
Result	74.3 ± 2.9	μg/dm^3

Excel file: exampl_5_9.xls

5.6 Calibration Uncertainty

A decisive majority of analytical measurements involve a calibration step, which is associated with the relative (comparative) character of measurements. At the calibration step, a calibration curve technique is usually used, which is determined using linear regression. This step of the analytical procedure has an influence on the combined uncertainty of the determination result for the real sample. Standard uncertainty due to that step of the analytical procedure should be included in the uncertainty budget.

There are four sources of uncertainty due to the calibration step which can influence the standard uncertainty of a single measurement $u_{(x_{smpl})}$ [9, 18–20]:

- repeatability of reading the value of a signal y both for standard samples (based on measurements for which the calibration curve is determined) and for study samples – $u_{(x_{smpl},y)}$,
- uncertainty due to the determination of the reference value for standard samples $-u_{(x_{smpl},x_{std_i})}$,
- the influence of the manner of preparing the standard samples, usually using a method of consecutive dilutions,
- incorrect approximation of measurement points using a regression curve.

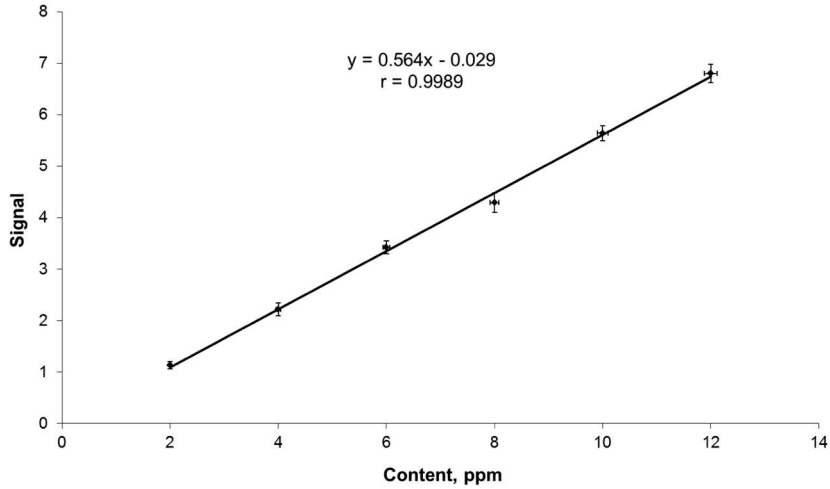


Figure 5.4 An example of a calibration graph along with the marked uncertainty values associated both with the reading of the signal values and the reference values.

Figure 5.4 presents an example of a calibration graph along with the marked uncertainty values associated both with the reading of the signal values and the reference values.

Using a calibration curve, drawn based on equations (1.63–1.68 in Chapter 1), it is possible to determine and identify the uncertainty of the determined regression curve through the determination of confidence intervals. Those intervals are determined using a correlation that is described by the following equation:

$$\Delta y_i = Y \pm SD_{xy} \cdot t_{(\alpha, f=n-2)} \sqrt{\frac{1}{n} + \frac{\left(x_i - x_m\right)^2}{Q_{xx}}} \tag{5.10}$$

where:

Δy_i – confidence interval of the calculated value Y for a given value x_i,

Y – values calculated based on the regression curve equation for given values x_i,

SD_{xy} – residual standard deviation,

$t_{(\alpha, f=n-2)}$ – Student's t test parameter,

n – the total number of standard samples used for the determination of the calibration curve (number of points),

x_i – value x for Δy_i is calculated,

x_m – mean value x (x is most frequently the analyte concentration and is the mean of all the concentrations of a standard solution for which the measurement was made in order to make a standard curve),

Q_{xx} – parameter calculated according to a relation described by the equation:

$$Q_{xx} = \sum_{i=1}^{n} \left(x_i - x_m \right)^2 \tag{5.11}$$

Standard uncertainty for x_{smpl} due to the uncertainty of calibration and linear regression method $u_{(x_{smpl},y)}$ may be calculated using the determined regression parameters according to the following relationship:

$$u_{(x_{smpl},y)} = \frac{SD_{xy}}{b} \sqrt{\frac{1}{p} + \frac{1}{n} + \frac{\left(x_{smpl} - x_m \right)^2}{Q_{xx}}} \tag{5.12}$$

where:

$u_{(x_{smpl},y)}$ – standard uncertainty for the determination of the x_{smpl} concentration due to the application of the determined calibration correlation,

b – the slope of the calibration curve,

p – the number of measurements (repetitions) carried out for a given sample.

Figure 5.5 presents a calibration curve along with the marked confidence intervals and the determined uncertainty value for the determination of an analyte's concentration in an examined sample.

The value of uncertainty for the determination of analyte concentration in the applied standard samples is usually significantly smaller compared to the uncertainty associated with the calculation of analyte concentration based on the determined calibration function:

$$u_{(x_{smpl},x_{std_i})} << u_{(x_{smpl},y)} \tag{5.13}$$

Therefore, its value may be estimated by only considering the number of standard samples used at the calibration stage. Because usually

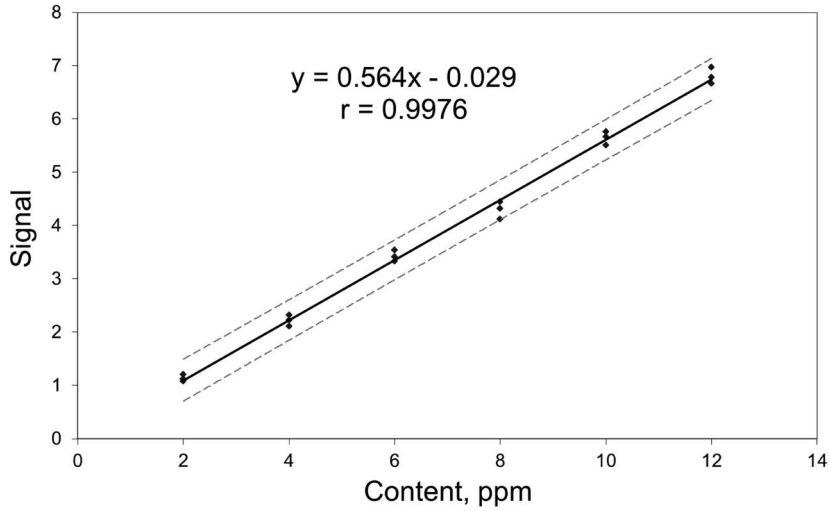


Figure 5.5 A calibration curve along with the marked confidence intervals and the determined uncertainty value for the determination of an analyte's concentration in an examined sample.

only one basic standard is used and then appropriate standard solutions are made (consecutive dilutions), standard uncertainty due to the application of standard solutions at the calibration step may be described by the following equation:

$$u_{\left(x_{smpl}, x_{std_i}\right)} \approx \frac{u_{\left(x_{std_i}\right)}}{n} \tag{5.14}$$

Such an uncertainty value does not allow for the uncertainty associated with the manner of standard sample preparation. If each standard sample is prepared by consecutive dilutions, then the uncertainty budget must allow for the standard uncertainties associated with the step of standard sample preparation. Usually, the standard uncertainty of a result, associated with an applied calibration technique, requires only the value $u_{\left(x_{smpl}, y\right)}$.

Example 5.10

Problem: A calibration curve was determined using determinations of analyte concentration in samples of six standard solutions, making three independent measurements for each of the solutions.

Calculate:

- regression parameters of the calibration curve,
- confidence intervals,
- uncertainty value of the determination value for the real sample due to calibration, for which three independent measurements were made and the result was calculated using the determined curve,

Data: results, ppm:

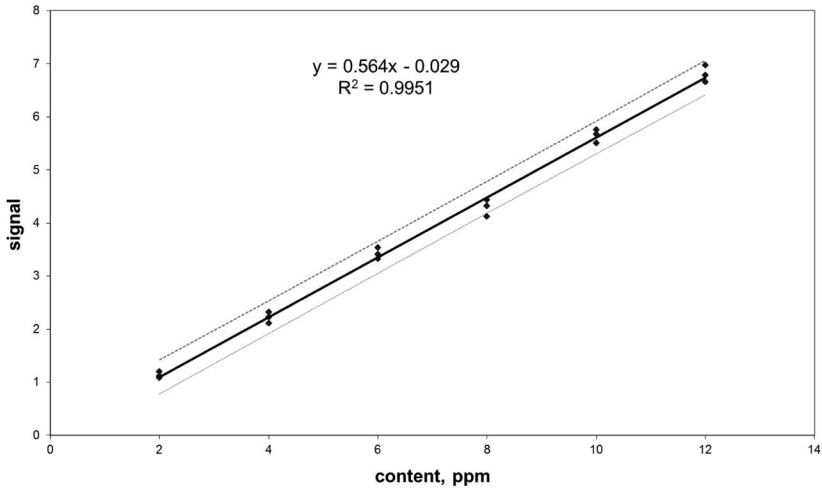
	DATA	
	X, PPM	*Y*, SIGNAL
1	2	1.12
2	2	1.2
3	2	1.08
4	4	2.11
5	4	2.32
6	4	2.23
7	6	3.33
8	6	3.54
9	6	3.41
10	8	4.12
11	8	4.32
12	8	4.44
13	10	5.67
14	10	5.76
15	10	5.51
16	12	6.97
17	12	6.78
18	12	6.66

Result for sample	7.59	ppm
Number of measurements for sample	3	

SOLUTION:

n	18
Slope – b	0.564
Intercept – a	–0.029
Residual standard deviation – $SD_{x,y}$	0.143
Regression coefficient – r	0.9976
Q_{xx}	210
Uncertainty for result due to calibration	0.16 ppm
Relative uncertainty for result due to calibration	2.1%
$t(\alpha = 0.05;\ f = n - 2)$ – from Table A.1	2.12

Graph:



Excel file: exampl_5_10.xls

5.7 Conclusion

Each analytical result derives from a conducted measurement. The ultimate goal for an analyst is to obtain a result that will most reliably reflect the expected (actual, real) value. The certainty of the analytical result depends on the uncertainties occurring at all the steps of an analytical procedure, the basic tool for any analyst.

The most crucial parameter affecting a measurement result's uncertainty is the parameter with the highest uncertainty value. Therefore, it is necessary to determine the sources and types of uncertainty for individual steps of an analytical procedure, and more exactly for each measurand. Combined uncertainty covers all sources of uncertainty that are relevant for all analyte concentration levels. It is a "key indicator" of both fitness-for-purpose and reliability of results.

Uncertainty is a basic property of each measurement. Uncertainty occurs always and at any step of a measurement procedure. Hence, it is not a property that should result in additional difficulties during the measurement procedure.

References

1. International vocabulary of metrology — Basic and general concepts and associated terms (VIM), Joint Committee for Guides in Metrology, JCGM 200, 2012.
2. ISO/IEC Guide 98-3:2008. Uncertainty of Measurement – Part 3: Guide to the Expression of Uncertainty in Measurement (GUM:1995).
3. ISO/IEC Guide 98-1:2024. Guide to the Expression of Uncertainty in Measurement (GUM) – Part 1: Introduction.
4. Williams A., Introduction to measurement uncertainty in chemical analysis, *Accred. Qual. Assur.*, 3, 92–94, 1998.
5. Populaire A., and Campos Gimenez E., A simplified approach to the estimation of analytical measurement uncertainty, *Accred. Qual. Assur.*, 10, 485–493, 2005.
6. Roy S., and Fouillac A.-M., Uncertainties related to sampling and their impact on the chemical analysis of groundwater, *Trends Anal. Chem.*, 23, 185–193, 2004.
7. Meyer V.R., Measurement uncertainty, *J. Chromatogr. A*, 1158, 15–24, 2007.
8. Kadis R., Evaluating uncertainty in analytical measurements: the pursuit correctness, *Accred. Qual. Assur.*, 3, 237–241, 1998.
9. Conti M.E., Muse O.J., and Mecozzi M., Uncertainty in environmental analysis: theory and laboratory studies, *Int. J. Risk. Assess. Manag.*, 5, 311–335, 2005.
10. Love J.L., Chemical metrology, chemistry and the uncertainty of chemical measurements, *Accred. Qual. Assur.*, 7, 95–100, 2002.
11. Armishaw P., Estimating measurement uncertainty in an afternoon. A case study in the practical application of measurement uncertainty, *Accred. Qual. Assur.*, 8, 218–224, 2003.
12. Konieczka P., The role of and place of method validation in the quality assurance and quality control (QA/QC) System, *Crit. Rev. Anal. Chem.*, 37, 173–190, 2007.
13. Sahuquillo A., and Rauret G., Uncertainty and traceability: the view of the analytical chemist, in: Fajgelj A., Belli M., and Sansone U. (eds.), Combining and Reporting Analytical Results, RSC, Springer, Berlin, 2007.
14. Taverniers I., Van Bockstaele E., and De Loose M., Trends in quality in the analytical laboratory. I. Traceability and measurement uncertainty of analytical results, *Trends Anal. Chem.*, 23, 480–490, 2004.
15. Ellison S.L.R., Rosslein M., and Williams A., EURACHEM, *Quantifying Uncertainty in Analytical Measurements*, second edition, 2000.
16. Ellison S.L.R., and Barwick V.J., Using validation data for ISO measurements uncertainty estimation. Part 1. Principles of an approach using cause and effect analysis, *Analyst*, 123, 1387–1392, 1998.

17. Kufelnicki A., Lis S., and Meinrath G., Application of cause-and-effect analysis to potentiometric titration, *Anal. Bioanal. Chem*., 382, 1652–1661, 2005.
18. Danzer K., and Currie L.A., Guidelines for calibration in analytical chemistry, *Pure Appl. Chem*., 70, 993–1014, 1998.
19. Bonate P.L., Concepts in calibration theory, part IV: prediction and confidence intervals, *LC-GC*, 10, 531–532, 1992.
20. Miller J.N., Basic statistical methods for analytical chemistry part 2. Calibration and regression methods, *Analyst*, 116, 3–14, 1991.

6
Reference Materials

6.1 Definitions [1, 2]

Reference Material (RM) – material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in the measurement or examination of nominal properties.

Certified Reference Material (CRM) – reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.

Homogeneity – condition of having a uniform structure or composition with respect to one or more specified properties. RM is said to homogeneous with respect to a specified property if the property value, as determined by tests on samples of specified size, is found to lie within the specified uncertainty limits, the samples being taken either from different supply units (bottles, packages, etc.) – between-bottle homogeneity, or from a single supply unit – within-bottle homogeneity.

Stability – ability of a reference material, when stored under specified conditions, to maintain a stated property value within specified limits for a specified period of time.

6.2 Introduction

Reference materials play a significant role in all the elements of the quality assurance system that evaluates the reliability of measurement results. The range of their application varies and includes [3–7]:

- validation of analytical procedures, where reference materials are used to determine precision and accuracy,
- interlaboratory comparisons, where they are applied as subject matter for studies,

- estimating the uncertainty of a measurement,
- documenting traceability.

With regard to the function that is played in a measurement process, RMs may be divided into pure substances, those that have a high and strictly defined level of purity, and standard solutions.

The general classification of RMs is presented in Figure 6.1 [8], and a detailed classification of RMs is presented in Table 6.1 [9].

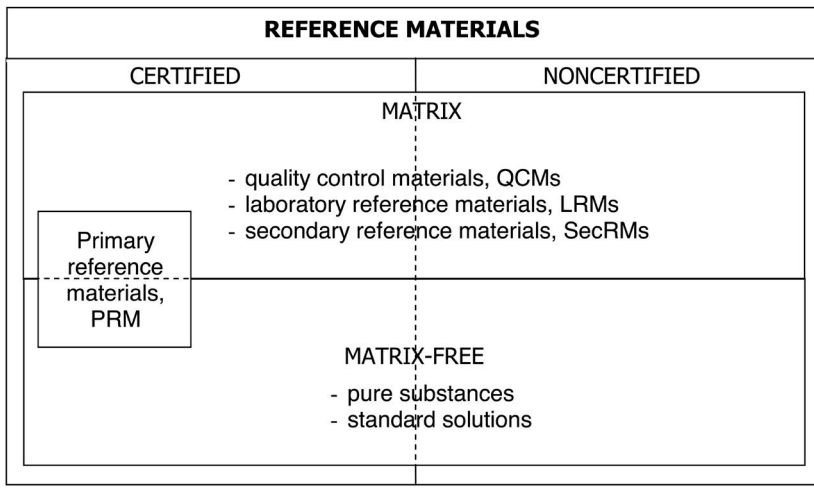


Figure 6.1 Classification of reference materials [8].

Table 6.1 Classification of Reference Materials Suitable for Chemical Investigations [9]

PARAMETER		ADDITIONAL REMARKS
Property	Chemical composition	RMs, being either pure chemical compounds or representative sample matrices, either natural or with added analytes (e.g. animal fats spiked with pesticides for residue analysis), characterized for one or more chemical or physicochemical property values
	Biological and clinical properties	Materials characterized for one or more biochemical or clinical property values
	Physical properties	Materials characterized for one or more physical property values, for example, melting point, viscosity, density
	Engineering properties	Materials characterized for one or more engineering property values (e.g. hardness, tensile strength or surface characteristics)

Table 6.1 (*Continued*) Classification of Reference Materials Suitable for Chemical Investigations [9]

PARAMETER		ADDITIONAL REMARKS	
	Miscellaneous	These principal categories are subdivided into subcategories as indicated in the following draft list. Other subcategories can be added at any time to address the needs of applicants seeking recognition of competence in producing types of reference materials not currently listed	
Chemical nature	Single major Constituent	High purity	Pure specific entity (isotope, element or compound) stochiometrically and isotopically certified in amount-of-substance ratios with total impurities <10 μmol/mol
		Primary chemicals	As above, but with limits of <100 μmol/mol
		Defined purity	As above, but with limits of <50 μmol/mol
	Matrix types	Major constituents	Major constituents (in matrix) >100 mmol/kg or >100 mmol/dm^3
		Minor constituents	Minor constituents (in matrix) <100 mmol/kg or <100 mmol/dm^3
		Trace constituents	Trace constituents <100 μmol/kg or <100 μmol/dm^3
		Ultra trace constituents	Ultra trace constituents <100 nmol/kg or <100 nmol/dm^3
Traceability	0 Primary class	Pure specified entity certified to SI at the smallest achievable uncertainty	
	I class	Certified by measurement against class 0 RM or SI with defined uncertainty (no measurable dependence on matrix)	
	II class	Verified by measurement against class I or 0 RM with defined uncertainty	
	III class	Described linkage to class 0, I, II	
	IV class	Described linkage other than to SI	
	V class	No described linkage	
Uncertainty of determination of analyte concentration	With uncertainty value	Primary reference materials (PRMs)	
		Certified reference materials (CRMs)	
	Without uncertainty value	Laboratory reference materials (LRMs)	
		Quality control materials (QCMs)	
Field of application		Validation of analytical method	
		Establishing measurement traceability	
		Calibrating an instrument	
		Assessment of a measurement uncertainty	
		Assessment of a measurement method	
		Recovery studies	
		Quality control	

Preparation of the RM involves the following:

- material selection,
- obtaining an appropriate amount of the material,
- selection and purchase of appropriate containers, labels, etc.,
- initial material preparation (grinding, sifting, appropriate fraction grain size),
- initial examination of the material's homogeneity,
- determination of main components,
- putting the materials into containers,
- final examination of the material's homogeneity,
- disinfection of the material (ensuring its biological stability),
- determining the humidity,
- organization of an interlaboratory comparison, in order to carry out a certification process,
- statistical analysis of the obtained results (rejection of deviating results, calculating means, standard deviations and the confidence intervals),
- determination of values attested to on the basis of hitherto formulated criteria, and printing the attestation certificate.

A general procedure for preparing RMs is shown schematically in Figure 6.2 [8].

RM can perform its function only when each of its users receives a material with exactly the same parameters. It may be achieved in two ways: by sending the same material sample or sending material samples with the same parameters (homogeneous, stable during storage, stable since the moment of production until their use) [9].

The selection of the RM depends on the needs at a given time, the type of analytical measurements in which it is going to be used, and its availability. No certified reference materials (laboratory reference material and material for quality control) and certified reference materials (primary reference material and certified reference material) differ in accuracy, precision and the uncertainty in the determination of given parameters.

That is why CRMs have a higher position in the "metrological hierarchy". The requirements at the production stage, according to ISO recommendations, are more rigorous, which is reflected in their price and thus their availability. Uncertified RMs, including the LRMs

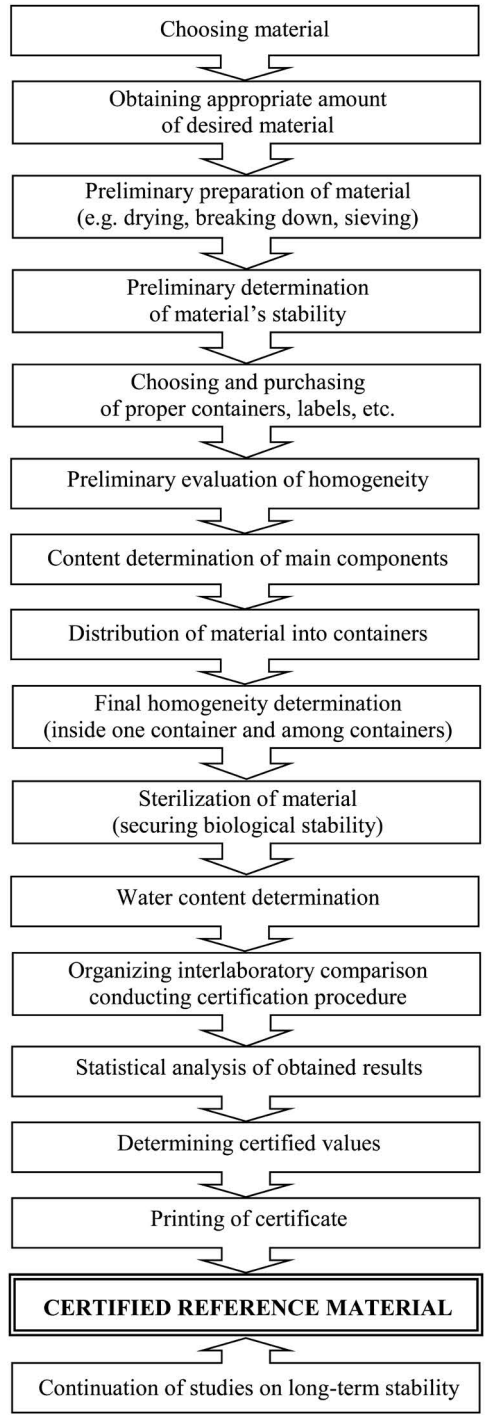


Figure 6.2 A general procedure for certified reference materials preparation – example for solid CRMs [8].

(cheaper and more available), are used mainly for the calibration of measuring instruments and checking analytical procedures [9, 10].

6.3 Parameters That Characterize RMs

6.3.1 General Information

The certification of RMs is something more than just performing a series of accurate and precise measurements traceable to SI standards or to any other metrological system. A certification process involves the preparation of a great number of homogeneous, stable and appropriately packaged samples, which are representative parts of a given production batch.

It is very important to pay special attention not only to the preparation of stable and homogeneous primary materials but also to sampling [11]. One should take into account microbiological degradation, which can be minimized by decreasing the content of water in the material to the level of 1%–3% of relative humidity. It is also recommended to pack the RM samples into appropriate containers in the argon atmosphere (bottles with fillers, penicillin vials or ampoules).

RMs should be prepared in such a way that they are homogeneous, stable and have constant characteristics over a sufficiently long period.

The parameters that characterize CRMs [12–18] are as follows:

- representativeness,
- homogeneity,
- stability,
- certified value.

6.3.2 Representativeness

Representativeness is a property that describes a similarity between individual samples with regard to:

- matrix composition,
- analyte concentration,
- manner of the connection between the analytes and the matrix,
- type and concentration of interfering substances,
- physical state of the material.

For practical reasons, the achievement of the required similarity is not always possible. A material should be homogeneous and stable, but in the process of homogenization and stabilization, a change may occur in the connection between the analyte and the matrix. In such cases, the user should be informed about the actual state of the material, the manner of processing and how to achieve a representative sample of the material for further analysis.

6.3.3 Homogeneity

Homogeneity study is a comparison of the obtained results for the random samples of the RM. It is carried out at the stage of distributing the RM into the appropriate containers.

There are two types of homogeneity [13]:

- within-bottle homogeneity,
- between-bottle homogeneity.

The influence of the *within-bottle* heterogeneity of the material on the result of the certified value may be eliminated by sampling a greater amount of the material. That is why it is necessary to define the minimum amount (mass) of the RM samples for the study.

A user has no influence upon the *between-bottle* heterogeneity of the material. This value should be determined by the producer of the RM and taken into account in the uncertainty budget of the certified value.

Both sources of heterogeneity of RMs are presented in Figure 6.3.

6.3.4 Stability

A stability study, next to the homogeneity study, plays a decisive role in the production of RMs. The stability of the RM is determined by using the analysis of the certified parameters in the samples of materials stored in a so-called reference temperature (with an assumption that in that temperature, the composition of the RM does not change) in relation to samples stored in temperatures recommended for a given RM.

During the storage and transportation, the RM is exposed to the influence of various external factors (temperature, light, oxygen,

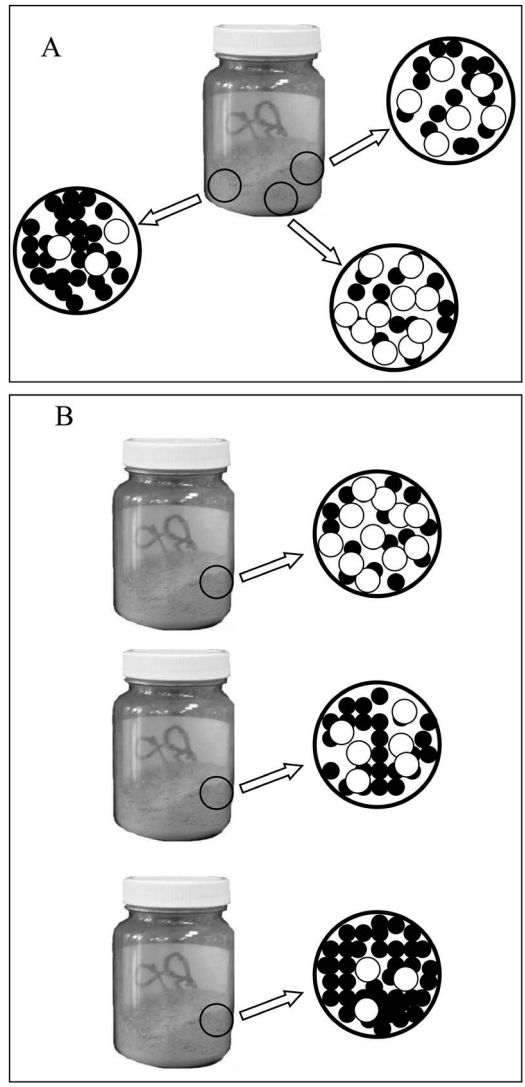


Figure 6.3 Sources of reference materials heterogeneity: A – within-bottle; B – between-bottle.

humidity, microbiological activity) that may affect its composition [16]. However, the value of a given parameter of the material should be stable over the whole validity period.

There are two types of RM stability: [14–16]

- long-term stability (e.g. shelf-life),
- short-term stability (e.g. stability during transportation).

Stability studies require the application of fast measurement methods, low-mass samples and the high repeatability of the measurements. The studies are carried out for various temperatures and storage durations.

Studying the stability of RMs may be considered in two aspects:

- classical (long term),
- isochronous.

In case of the classical stability study, stability is determined by comparing the results obtained for samples stored in the recommended conditions and for the reference samples, usually stored in a lower temperature, for example, −40°C.

Such studies are carried out a short time before the hitherto determined expiry date and may result in extending the validity period.

An isochronous stability study is based on deducing the stability of the RM on the basis of analyses of samples stored over a short period (several weeks) and at various temperatures (usually higher than the recommended storage temperature) [16].

6.3.5 Certified Value

RM certification is carried out according to the strictly determined rules, as described in an appropriate ISO Guides [19–22]. In contrast to pure substances and calibration solutions, matrix RMs cannot be certified using direct gravimetric measurement. In this case, an additional stage is required: a complete change or the removal of the matrix. Thus, the following solutions are applied [23]:

- measurements at a single laboratory, using the absolute methods, that is, methods that give the results directly in units of measurement or methods that allow the result to be expressed in those units through the application of mathematical equations from the appropriate physical and chemical theories,
- measurements at a single laboratory using two or more methods, by two or more analysts,
- interlaboratory studies using one or several various methods, including the absolute methods.

It must be remembered that certification studies should be carried out by the laboratories with supreme and proven competence.

Certification is based on material sample analyses, using one or more methods at one or several laboratories, in which each of the measurement series is carried out with the highest accuracy and traceability, and must be documented by a complete uncertainty budget.

The aim of material certification is to ascribe certain values of individual properties to a group or individual units. The reliability of the obtained results of analytical measurements is a self-evident condition, essential for certification [24].

The final uncertainty value of the CRM, according to the guidelines presented by Guide to the Expression of Uncertainty in Measurement [25, 26], should include all the uncertainty sources described in the following equation [27]:

$$u_{CRM} = \sqrt{u_{cert}^2 + u_{bott}^2 + u_{ls}^2 + u_{ss}^2} \tag{6.1}$$

where:

u_{cert} – uncertainty of determining the certified value,
u_{bott} – uncertainty associated with the within-bottle homogeneity,
u_{ls} – uncertainty associated with the long-term stability,
u_{ss} – uncertainty associated with the short-term stability.

6.4 Production of CRMs – Requirements (ISO 17034)

Reference Material Producer (RMP) must meet a number of requirements set out in ISO 17034 [22] in order to prove their technical competence and the reliability of the materials they produce.

The main areas covered by ISO 17034 that must be met by RMPs relate to:

a. **technical competence** relating to the qualification of personnel, equipment, analytical methods and their validation:
   - personnel involved in CRM production must have the appropriate qualifications and experience and should regularly participate in training to keep up to date with new methods and technologies,
   - the RMP must regularly assess the competence of the staff, ensuring their ability to perform the assigned tasks,

b. **quality management**, which includes management systems, documentation, supervision of activities and handling deviations and complaints
   - the RMP must have a well-documented quality management system, including policies, procedures and work instructions,
   - the RMP must supervise documentation to prove its regular review, updating and appropriate archiving,
   - the RMP must manage risk, that is, identify those elements of the production process that may affect the quality of CRM,
   - the RMP must regularly conduct internal audits to assess the effectiveness of the quality management system and compliance with ISO 17034,
   - management should regularly review the quality management system to ensure its continuous improvement,

c. **resources for the production of reference materials**, including rules on the processes for the production, identification, storage and distribution of reference materials:
   - the RMP must have the appropriate equipment and infrastructure to ensure that CRMs are manufactured in accordance with quality requirements, the equipment used must be regularly calibrated and maintained, and the calibration results should be properly documented,
   - the CRM production process itself must be carefully planned and controlled,
   - it is very important to ensure that the reference materials are stable, homogeneous and consistent with the declared properties,

d. **monitoring and validation**, that is ensuring that each CRM is accurately monitored from the production stage to the final product:
   - the RMP must ensure that each stage of production is monitored, controlled and documented to enable CRM traceability,
   - all methods used in CRM production must be validated to confirm that they are fit for purpose,

- the RMP must accurately characterize the CRM, specifying the reference values and their uncertainties,
- CRMs must be tested for their homogeneity and stability to confirm that the materials are uniform and stable for a certain period of time,
- the CRM certification process must be carried out according to recognized methods, and the results must be documented in the form of a certificate,
- RMP must have procedures in place to handle complaints regarding CRM, ensuring a quick response to reported problems,
- if inconsistencies are detected in the production process or in the CRM itself, RMP must take appropriate corrective and preventive actions.

The above requirements are designed to ensure that RMPs provide reference materials of the highest quality, which are necessary for precise measurements and research in various fields of science and industry.

6.5 Practical Application of CRMs

These are the main issues associated with the application of the CRMs [24–31]:

- determination of validation parameters – first of all their precision and accuracy,
- examining the skills of an analyst or a laboratory,
- routine control of precision and accuracy of the performed determinations,
- laboratory accreditation,
- the quality control of performance of a given laboratory,
- estimating measurement uncertainty,
- monitoring and ensuring traceability,
- calibration of measuring instruments.

It is not possible to prepare appropriate CRMs for all the analytical tasks, due to the high heterogeneity of matrix compositions and the wide spectrum of analytes present in the examined samples. A

good knowledge of analytical procedures and the available materials is, therefore, a key to the right choice.

The selection of the RM should allow for the following criteria:

- availability (the issue of the matrix composition),
- concentration range of the reference value,
- uncertainty value of the reference value,
- traceability of the reference value,
- required uncertainty value of the measurement,
- influence of the CRM uncertainty on the combined uncertainty of the measurement,
- quality of the CRM producer (competence, reputation),
- composition of the sample matrix,
- price.

Detailed information concerning the CRMs, and help in finding an appropriate CRM, can be found in the following databases available at the Internet websites (accessed on 28.08.2024):

http://www.comar.bam.de
https://nucleus.iaea.org/rpst/ReferenceProducts/About/index.htm
https://crm.jrc.ec.europa.eu/
https://www.nist.gov/srm
https://www.jctlmdb.org/#/app/home

Using CRMs requires compliance with the rules of good laboratory practice at laboratories that determine the trace components in the examined samples:

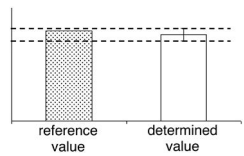
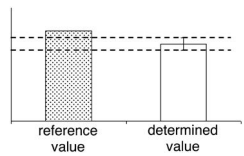
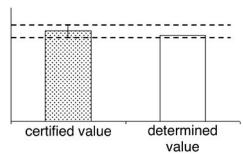
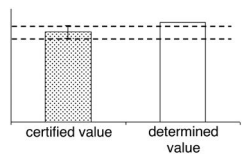
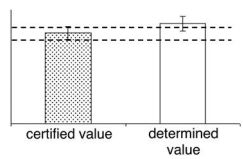
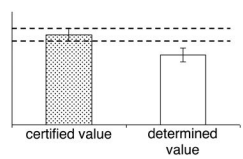
- it is necessary to comply with the recommendations of the RM producer, for example, concerning the minimum mass of the RM sampled, the validity period and the manner of storage,
- it is necessary to determine the concentration of water (in case of solid materials) for the RM samples taken simultaneously with the RM sample for the study,
- the taken and nonused RM cannot be replaced into container.

RMs are an essential tool for the determination of accuracy and/or precision. Because one of the main problems associated with this process is the interpretation and numerical presentation of the

determined parameter, this book presents the basic formulas and correlations that help in selecting the manner of documenting the values of the determined parameters.

It seems practical to provide a graphical comparison of the reference (certified) value with the value obtained during the measurement (determined one). Possible situations, depending on the information on the two compared values, together with the associated conclusions are presented in Table 6.2.

Table 6.2 A Suitable Way of Graphically Comparing the Reference (Certified) Value with the Determined Value

CONDITIONS	GRAPHICAL PRESENTATION	CONCLUSIONS
Reference value without providing the uncertainty (not a certified value) and determined value with a provided uncertainty		Determined value agreed with the reference value
		Conclusion impossible
Reference value with the uncertainty and determined value without a provided uncertainty		Determined value agreed with the reference value
		Conclusion impossible
Reference value with the uncertainty and determined value with a provided uncertainty		Determined value agreed with the certified value
		Determined value not agreed with the certified value

Example 6.1

Problem: Five independent determinations of total mercury were carried out for the samples of the certified reference material NRCC-DORM-2 – dogfish muscle.

The certified value given by the producer is 4.64 ± 0.28 μg/g.

Using a graphical method, test the agreement of the obtained value with the certified value.

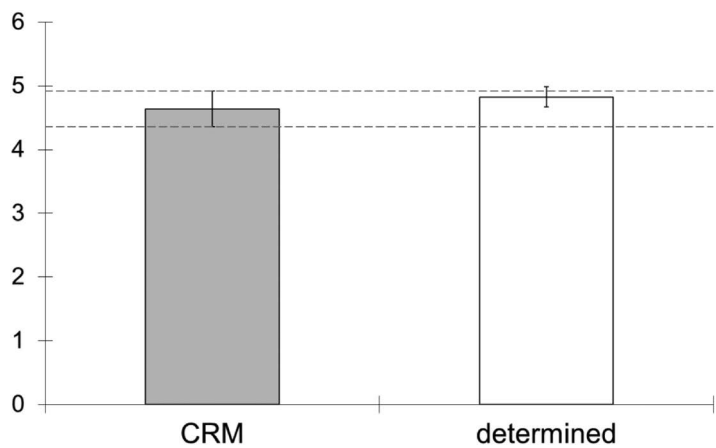
Data: result series, μg/g:

1	4.76
2	4.57
3	4.94
4	5.04
5	4.82

SOLUTION:

Mean, μg/g	4.83
SD, μg/g	0.18
$U\,(k=2)$, μg/g	0.16

Graph:



Conclusion: An obtained value agreed with certified one.

Excel file: exampl_6_1.xls

Example 6.2

Problem: Six independent determinations of total mercury were carried out for the samples of the reference material GBW 07601 – powdered human hair.

The assigned value given by the producer is 0.36 μg/g.

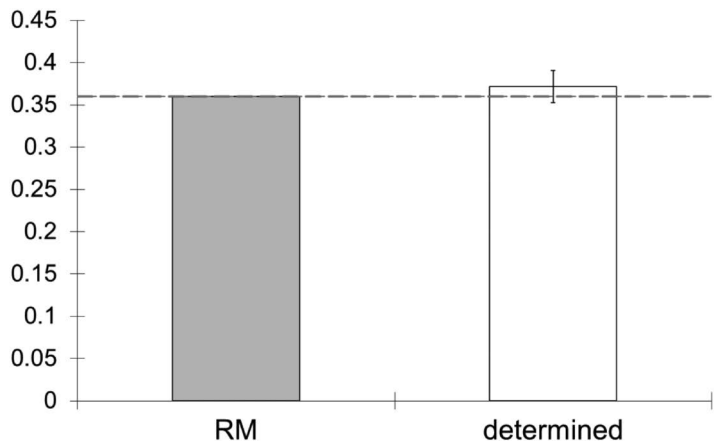
Using a graphical method, test the agreement of the obtained value with the assigned value.

Data: result series, μg/g:

1	0.38
2	0.34
3	0.35
4	0.39
5	0.37
6	0.40

SOLUTION:

Mean, μg/g	0.372
SD, μg/g	0.023
$U\,(k=2)$, μg/g	0.019



Conclusion: An assigned value is in the range of obtained value ± uncertainty.

Excel file: exampl_6_2.xls

An alternative solution is to determine the conformity of the reference value with the determined value using appropriate tests. The following options are feasible:

1. A comparison of the standard deviation values in the series of measurements for *CRM*, with the value of expanded uncertainty for *CRM*, and the comparison of the determined values with the certified value.

   The following condition must be fulfilled:

$$\frac{SD_{det}}{\sqrt{n}} < U_{CRM} \tag{6.2}$$

   where:

   SD_{det} – standard deviation for the measurement series for *CRM*,

   n – the number of measurements for *CRM*,

   U_{CRM} – the expanded uncertainty for *CRM*.

   and

$$x_{CRM} - U_{CRM} < x_{det} < x_{CRM} + U_{CRM} \tag{6.3}$$

   where:

   x_{det} – determined value,

   x_{CRM} – certified value.

Example 6.3

Problem: Five independent determinations of total mercury were carried out for the samples of the certified reference material NRCC-DORM-2 – dogfish muscle.

The certified value given by the manufacturer is 4.64 ± 0.28 μg/g.

Using the aforementioned method, test the agreement of the obtained value with the certified value.

Data: result series, μg/g:

1	4.76
2	4.57
3	4.94
4	5.04
5	4.82

SOLUTION:

x_{det}, μg/g	4.83
SD_{det}, μg/g	0.18
n	5
$\frac{SD_{det}}{\sqrt{n}}$, μg/g	0.080
U_{CRM}, μg/g	0.28
x_{CRM}, μg/g	4.64
$x_{CRM} - U_{CRM}$, μg/g	4.36
$x_{CRM} + U_{CRM}$, μg/g	4.92

$$\boxed{\frac{SD_{det}}{\sqrt{n}} < U_{CRM}}$$

$$\boxed{x_{CRM} - U_{CRM} < x_{det} < x_{CRM} + U_{CRM}}$$

Conclusion: An obtained value agreed with certified one.

Excel file: exampl_6_3.xls

Example 6.4

Problem: Four independent determinations of lead were carried out for the samples of the certified reference material NIST-SRM 1633b – coal fly ash.

The certified value given by the producer is 68.2 ± 1.4 μg/g.

Using the aforementioned method, test the agreement of the obtained value with the certified value.

Data: result series, μg/g:

1	70.2
2	71.4
3	69.8
4	70.6

SOLUTION:

x_{det}, µg/g	70.5
SD_{det}, µg/g	0.68
n	4
$\frac{SD_{det}}{\sqrt{n}}$, µg/g	0.34
U_{CRM}, µg/g	1.4
x_{CRM}, µg/g	68.2
$x_{CRM} - U_{CRM}$, µg/g	66.8
$x_{CRM} + U_{CRM}$, µg/g	69.6

$$\boxed{\frac{SD_{det}}{\sqrt{n}} < U_{CRM}}$$

$$\boxed{x_{CRM} - U_{CRM} < x_{det} < x_{CRM} + U_{CRM}}$$

Conclusion: An obtained value no agreed with certified one.

Excel file: exampl_6_4.xls

2. Application of Student's *t* test.
   The value of the parameter *t* is calculated according to the formula:

$$t = \frac{|x_{det} - x_{CRM}|}{SD_{det}}\sqrt{n} \tag{6.4}$$

The calculated value should be compared with the critical value from the distribution values for an appropriate significance level (α) and the number of degrees of freedom $f = n - 1$.

The formula (6.4) does not allow for the uncertainty of the certified value; that is why it is recommended to use its modified version:

$$t = \frac{|x_{det} - x_{CRM}|}{\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}}\sqrt{n} \tag{6.5}$$

where:

$u_{(x_{det})}$ – combined uncertainty of the determined value,
$u_{(x_{CRM})}$ – combined uncertainty of the certified value.

3. The comparison of the certified value with the determined value, including the uncertainties for both the values.

The following correlations are examined:

$$|x_{det} - x_{CRM}| < 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}} \tag{6.6}$$

$$|x_{det} - x_{CRM}| \geq 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}} \tag{6.7}$$

Satisfying the first relation implies conformity of the determined value with the certified value, and satisfying the second relation denotes the lack of conformity between these values.

Example 6.5

Problem: Five independent determinations of total mercury were carried out for the samples of the certified reference material NRCC-DORM-2 – dogfish muscle.

The certified value given by the manufacturer is 4.64 ± 0.28 μg/g.

Using the aforementioned method, test the agreement of the obtained value with the certified value.

Data: result series, μg/g:

1	4.76
2	4.57
3	4.94
4	5.04
5	4.82

SOLUTION:

x_{det}	4.83
SD_{det}	0.18
n	5
$u_{(x_{det})}$	0.080
$u_{(x_{CRM})}$	0.14
$\lvert x_{det} - x_{CRM}\rvert$	0.19
$2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}$	0.32

$$\boxed{|x_{det} - x_{CRM}| < 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}}$$

$$\left|x_{det} - x_{CRM}\right| \geq 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}$$

Conclusion: An obtained value agreed with certified one.

Excel file: exampl_6_5.xls

Example 6.6

Problem: Four independent determinations of lead were carried out for the samples of the certified reference material NIST-SRM 1633b – coal fly ash.

The certified value given by the producer is 68.2 ± 1.4 μg/g.

Using the aforementioned method, test the agreement of the obtained value with the certified value.

Data: result series, μg/g:

1	70.2
2	71.4
3	69.8
4	70.6

SOLUTION:

x_{det}	70.5
SD_{det}	0.68
n	4
$u_{(x_{det})}$	0.34
$u_{(x_{CRM})}$	0.70
$\left\|x_{det} - x_{CRM}\right\|$	2.3
$2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}$	1.6

$$\left|x_{det} - x_{CRM}\right| < 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}$$

$$\left|x_{det} - x_{CRM}\right| \geq 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{CRM})}}$$

Conclusion: An obtained value no agreed with certified one.

Excel file: exampl_6_6.xls

4. The application of *Z*-Score

The value of the *Z*-Score is calculated using the following formula:

$$Z = \frac{x_{det} - x_{CRM}}{s} \tag{6.8}$$

where:

s – the value of a deviation unit, which can be calculated as the combined uncertainty of the certified value and the determined value.

The reasoning is carried out using the following relations:

- if $|Z| \leq 2$, then the determined value agreed with the reference value,
- if $|Z| > 2$, then the determined value does not agree with the reference value.

Trueness value, due to application of *CRMs*, can be presented as recovery and should be calculated according the following equations:

$$\%R = \frac{x_{det}}{x_{CRM}}[\%] \tag{6.9}$$

$$U = k \cdot \frac{\sqrt{\left(u^2_{(x_{det})} + u^2_{(x_{CRM})}\right)}}{\left(\frac{x_{det} + x_{CRM}}{2}\right)}[\%] \tag{6.10}$$

The reasoning should be based on the following:
if the range $\%R \pm U$ includes value 100%, calculated value of trueness is acceptable.
The value of trueness is usually given as:

$$Trueness = \%R \pm U \tag{6.11}$$

and most frequently is expressed in %.

Example 6.7

Problem: Five independent determinations of total mercury were carried out for the samples of the certified reference material NRCC-DORM-2 – dogfish muscle.

The certified value given by the manufacturer is 4.64 ± 0.28 μg/g.

Using the obtained result, calculate trueness as a recovery value for $k = 2$.

Data: result series, μg/g:

1	4.76
2	4.57
3	4.94
4	5.04
5	4.82

SOLUTION:

x_{det}	4.83
x_{CRM}	4.64
SD_{det}	0.18
n	5
$u_{(x_{det})}$	0.080
$u_{(x_{CRM})}$	0.14
k	2
%R	**104.0%**
U	**6.8%**

$$\%R = \frac{x_{det}}{x_{CRM}} \cdot 100\%$$

$$U = k \cdot \frac{\sqrt{\left(u^2_{(x_{det})} + u^2_{(x_{CRM})}\right)}}{\left(\frac{x_{det} + x_{CRM}}{2}\right)}$$

Conclusion: A value of 100% is in the range of calculated trueness value.

Excel file: exampl_6_7.xls

Due to a limited number of certified reference materials, a widely known standard addition method is applied as an alternative manner of determining trueness.

The recovery is calculated based on increasing the signal (recalculated for concentration, content) after standard addition.

It is very important to fulfill requirements for that method, so increasing of the signal should be more than 50% of the value for sample and less than 150% of that value. The volume of the standard added should be negligible compare to the sample volume (no influence on matrix composition).

Example 6.8

Problem: Standard addition method has been used for the determination of trueness. Two series were conducted – for the real sample and for the sample with standard addition.

Using the obtained result, calculate trueness as a recovery value for $k = 2$. Assume the value $\alpha = 0.05$.

Data: results series, mg/dm^3:

	DATA	
	SAMPLE	SAMPLE WITH STANDARD ADDITION
1	33.54	57.03
2	33.11	58.11
3	32.87	59.03
4	33.75	57.88
5	34.39	58.23
6	33.33	60.34
7	32.05	57.99

			U	*k*
Standard concentration x_{st}	5000	mg/dm^3	5	2
Standard volume V_{st}	0.50	cm^3	0.02	2
Sample volume V_{smpl}	100.0	cm^3	0.2	2

SOLUTION:

Checking for outliers, using Dixon-Q test

	SAMPLE	SAMPLE WITH STANDARD ADDITION
No. of results – n	7	7
Range – R	2.34	3.31
Q_1	0.350	0.257
Q_n	0.274	0.396
Q_{crit}	0.507	0.507

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, for both series, there are no outliers in the results series. The calculated values of x_m, SD, CV and $u_{r(det)}$:

	SAMPLE	SAMPLE WITH STANDARD ADDITION	
X_m	33.29	58.37	mg/dm^3
SD	0.73	1.0	mg/dm^3
CV	2.2	1.8	%
$u_{r(det)}$	0.83	0.68	%

where $u_{r(det)}$ has been calculated as:

$$u_{r(det)} = \frac{CV}{\sqrt{n}}$$

The theoretical concentration after standard addition has been calculated according to formula:

$$x_{teor} = \frac{x_{m(smpl)} \times V_{smpl} + x_{st} \times V_{st}}{V_{smpl} + V_{st}}$$

Theoretical concentration after standard addition x_{theor}	58.00	mg/dm^3

The calculations of concentration increasing have been done as:

$$\Delta x_{theor} = x_{theor} - x_{smpl}$$

$$\Delta x_{det} = x_{smpl+st} - x_{smpl}$$

CONCENTRATION INCREASING		
Theoretical	Determined	
Δx_{theor}	Δx_{det}	
24.71	25.08	mg/dm^3

Before calculating recovery, it is necessary to check if the amount of standard added fulfilled a requirement for application of the standard addition method.

For that, both relations have to be fulfilled:

$$0.5 \times x_{det} < \Delta x_{theor} < 1.5 \times x_{det}$$

For the data:

$$16.65 < 24.71 < 49.93$$

Recovery is calculated as:

$$\%R = \frac{\Delta x_{det}}{\Delta x_{theor}}$$

And its expanded uncertainty for the value for $k = 2$ is calculated according to the following formula:

$$U(k=2) = 2 \cdot \%R \cdot \sqrt{u^2_{r(det)smpl} + u^2_{r(det)smpl+st} + \left(\frac{\frac{U_{x_{st}}}{k}}{x_{st}}\right)^2 + \left(\frac{\frac{U_{V_{st}}}{k}}{V_{st}}\right)^2 + \left(\frac{\frac{U_{V_{smpl}}}{k}}{V_{smpl}}\right)^2}$$

%R	101.5%
$\boldsymbol{U(k=2)_{\%R}}$	4.6%

A value of 100% is in the range of calculated trueness value, and there is no need to correct the results on bias.

Conclusion: The investigated method is accurate.

Excel file: exampl_6_8.xls

Example 6.9

Problem: Standard addition method has been used for the determination of trueness. Two series were conducted – for the real sample and for the sample with standard addition.

Using the obtained result, calculate trueness as a recovery value for $k = 2$. Assume the value $\alpha = 0.05$.

Data: results series, mg/dm^3:

	DATA	
	SAMPLE	SAMPLE WITH STANDARD ADDITION
1	53.23	110.1
2	54.87	111.6
3	55.98	108.1
4	51.34	121.5
5	50.21	118.1
6	56.11	109.9
7	53.88	115.3

			U	*k*
Standard concentration x_{st}	5000	mg/dm^3	5	2
Standard volume V_{st}	1.30	cm^3	0.02	2
Sample volume V_{smpl}	100.0	cm^3	0.2	2

SOLUTION:

Checking for outliers, using Dixon-*Q* test

	SAMPLE	SAMPLE WITH STANDARD ADDITION
No. of results – *n*	7	7
Range – *R*	2.34	3.31
Q_1	0.192	0.134
Q_n	0.022	0.254
Q_{crit}	0.507	0.507

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, for both series, there are no outliers in the results series. The calculated values of x_m, *SD*, *CV* and $u_{r(det)}$:

	SAMPLE	SAMPLE WITH STANDARD ADDITION	
X_m	53.66	113.51	mg/dm^3
SD	2.2	4.9	mg/dm^3
CV	4.2	4.3	%
$u_{r(det)}$	1.6	1.6	%

where $u_{r(det)}$ has been calculated as:

$$u_{r(det)} = \frac{CV}{\sqrt{n}}$$

The theoretical concentration after standard addition has been calculated according the following formula:

$$x_{teor} = \frac{x_{m(smpl)} \times V_{smpl} + x_{st} \times V_{st}}{V_{smpl} + V_{st}}$$

Theoretical concentration after standard addition x_{theor}	117.14	mg/dm^3

The calculations of concentration increasing have been done as:

$$\Delta x_{theor} = x_{theor} - x_{smpl}$$

$$\Delta x_{det} = x_{smpl+st} - x_{smpl}$$

CONCENTRATION INCREASING		
Theoretical	Determined	
Δx_{theor}	Δx_{det}	
63.48	59.85	mg/dm^3

Before calculating recovery, it is necessary to check if the amount of standard added fulfilled a requirement for application of the standard addition method.

For that, both relations have to be fulfilled:

$$0.5 \times x_{det} < \Delta x_{theor} < 1.5 \times x_{det}$$

For the data:

$$26.83 < 63.48 < 80.49$$

Recovery is calculated as:

$$\%R = \frac{\Delta x_{det}}{\Delta x_{theor}}$$

And its expanded uncertainty for value for $k = 2$ is calculated according to the following formula:

$$U(k=2) = 2 \cdot \%R \cdot \sqrt{u^2_{r(det)smpl} + u^2_{r(det)smpl+st} + \left(\frac{\frac{U_{x_{st}}}{k}}{x_{st}}\right)^2 + \left(\frac{\frac{U_{V_{st}}}{k}}{V_{st}}\right)^2 + \left(\frac{\frac{U_{V_{smpl}}}{k}}{V_{smpl}}\right)^2}$$

%R	94.3%
$\boldsymbol{U(k=2)_{\%R}}$	4.5%

A value of 100% is out of the range of calculated trueness value, and it is necessary to correct the results on bias.

Conclusion: The investigated method is not accurate.

Excel file: exampl_6_9.xls

6.6 Conclusion

The production and certification of RM are very costly, which is why the application of CRMs is usually limited to the verification of analytical procedures and only in some exceptional case to calibration (in comparative methods). Due to financial limitations, it is not recommended to use certified reference materials for a routine intralaboratory statistical control, nor in the interlaboratory comparisons. It is recommended, however, in competence tests.

CRMs play a crucial role in the system of estimation, monitoring and ensuring the quality of analytical measurement results. Their application, as noted above, is necessary in any laboratory. However, it must be said that using *CRM* at a laboratory does not automatically ensure the obtainment of reliable results. RMs must be applied in a rational way and do not nullify the remaining elements of the quality system.

RMs should be stored in conditions that guarantee the stability of their composition over the whole period of use.

References

1. International vocabulary of metrology — Basic and general concepts and associated terms (VIM), Joint Committee for Guides in Metrology, JCGM 200, 2012.
2. ISO Guide 80:2014. Guidelines for the in-house preparation of quality control materials (QCMs).
3. Emons H., Linsinger T.P.J., and Gawlik B.M., Reference materials: terminology and use. Can't one see the forest for the trees?, *Trends Anal. Chem*., 23(6), 442–449, 2004.
4. Rasberry S.D., Reference materials in the world of tomorrow, *Fresenius J. Anal. Chem*., 360, 277–281, 1998.
5. Lipp M., Reference materials – an industry perspective, *Accred. Qual. Assur*., 9, 539–542, 2004.
6. Pauwels J., and Lamberty A., CRMs for the 21st century: new demands and challenges, *Fresenius J. Anal. Chem*., 370, 111–114, 2001.
7. Majcen N., A need for clearer terminology and guidance in the role of reference materials in method development and validation, *Accred. Qual. Assur*., 8, 108–122, 2003.
8. Konieczka P., The role of and place of method validation in the quality assurance and quality control (QA/QC) system, *Crit. Rev. Anal. Chem*., 37, 173–190, 2007.
9. Konieczka P., and Namieśnik J. (eds.), Kontrola i zapewnienie jakości wyników pomiarów analitycznych, WNT, Warsaw, 2017 (*in Polish*).
10. Fellin P., and Otson R., A test atmosphere generation system for particle-bound PNA: development and use for evaluation of air sampling methods, *Chemosphere*, 27, 2307–2315, 1993.
11. Kramer G.N., and Pauwels J., The preparation of biological and environmental reference materials, *Mikrochim. Acta*., 123, 87–93, 1996.
12. Linsinger T.P.J., Pauwels J., Van der Veen A.M.H., Schimmel H., and Lamberty A., Homogeneity and stability of reference materials, *Accred. Qual. Assur*., 6, 20–25, 2001.
13. Van der Veen A.M.H., Linsinger T., and Pauwels J., Uncertainty calculations in the certification of reference materials. 2. Homogeneity study, *Accred. Qual. Assur*., 6, 26–30, 2001.
14. Van der Veen A.M.H., Linsinger T.P.J., Lamberty A., and Pauwels J., Uncertainty calculations in the certification of reference materials. 3. Stability study, *Accred. Qual. Assur*., 6, 257–263, 2001.
15. Pauwels J., Lamberty A., and Schimmel H., Quantification of the expected shelf-life of certified reference materials, *Fresenius J. Anal. Chem*., 361, 359–361, 1998.
16. Lamberty A., Schimmel H, and Pauwels J., The study of the stability of reference materials by isochronous measurements, *Fresenius J. Anal. Chem*., 360, 395–399, 1998.
17. Van der Veen A.M.H., and Pauwels J., Uncertainty calculations in the certification of reference materials. 1. Principles of analysis of variance, *Accred. Qual. Assur*., 5, 464–469, 2000.

18. Van der Veen A.M.H., Linsinger T.P.J., Schimmel H., Lamberty A., and Pauwels J., Uncertainty calculations in the certification of reference materials. 4. Characterisation and certification, *Accred. Qual. Assur.*, 6, 290–294, 2001.
19. ISO Guide 30:2015. Reference materials – selected terms and definitions.
20. ISO 33401:2024. Reference materials – contents of certificates, labels and accompanying documentation.
21. ISO 33405:2024. Reference materials – Approaches for characterization and assessment of homogeneity and stability.
22. ISO 17034:2016. General requirements for the competence of reference material producers.
23. Uriano G.A., and Gravatt C.C., The role of reference materials and reference methods in chemical analysis, *Crit. Rev. Anal. Chem.*, 6, 361–411, 1977.
24. Linsinger T.P.J., Pauwels J., Schimmel H., Lamberty A., Veen A.M.H., Schumann G., and Siekmann L., Estimation of the CRMs in accordance with GUM: application to the certification of four enzyme CRMs, *Fresenius J. Anal. Chem.*, 368, 589–594, 2000.
25. ISO/IEC Guide 98–3:2008. Uncertainty of measurement – Part 3: guide to the expression of uncertainty in measurement (GUM:1995).
26. ISO/IEC Guide 98-1:2024. Guide to the expression of uncertainty in Measurement (GUM) – Part 1: introduction.
27. Pauwels J., Van der Veen A., Lamberty A., and Schimmel H., Evaluation of uncertainty of reference materials, *Accred. Qual. Assur.*, 5, 95–99, 2000.
28. Caroli S., Forte G., and Iamiceli A.L., ICP-AES and ICP-MS quantification of trace elements in the marine macroalga fucus sample, a new candidate certified reference material, *Microchem. J.*, 62, 244–250, 1999.
29. Sutherland R.A., and Tack F.M.G., Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimized BCR sequential extraction procedure, *Anal. Chim. Acta*, 454, 249–257, 2002.
30. Caroli S., Senofonte O., Caimi S., Robouch P., Pauwels J., and Kramer G.N., Certified reference materials for research in Antarctica: the case of marine sediment, *Microchem. J.*, 59, 136–143, 1998.
31. Dybczyński R., Danko B., and Polkowska-Motrenko H., Some difficult problems still existing in the preparation and certification of CRMs, *Fresenius J. Anal. Chem.*, 370, 126–130, 2001.

7
Interlaboratory Comparisons

7.1 Definitions [1]

Interlaboratory comparisons – organization, performance and evaluation of tests on the same or similar test items by two or more laboratories in accordance with predetermined conditions.

Proficiency testing – determination of laboratory testing performance by means of interlaboratory comparisons.

Certification study – a study which assigns a reference value to a given parameter (e.g. analyte concentration) in a tested material or a given sample, usually with a determined uncertainty.

Method-performance study – interlaboratory research in which all participants act according to the same protocol and using the same test procedures to determine the characteristic features in a batch of identical test samples.

7.2 Introduction

Demand for results as a source of reliable analytical information poses new challenges for analytical laboratories: they need to be especially careful in documenting the results and the applied research methods. Ensuring a suitable quality of analytical results is essential because of the negative implications of presenting unreliable measurement results. The way to realize this goal is to implement a suitable quality assurance system at a laboratory through constant monitoring of the reliability of the analytical results and calibration. One of the most crucial means of that monitoring is participation in various interlaboratory studies [2].

Participation in these programs gives a chance for a laboratory to compare its results with those obtained by other laboratories and to prove its competence, which can be especially significant for laboratories with accreditation or those applying for accreditation. Moreover,

participation in analytical interlaboratory comparative studies gives a laboratory a chance to search and detect unexpected errors using comparisons with external standards and its own previous results, and in the case of error detection, undertake rectifying action [3].

A generalized scheme for conducting interlaboratory studies is shown in Figure 7.1 [4].

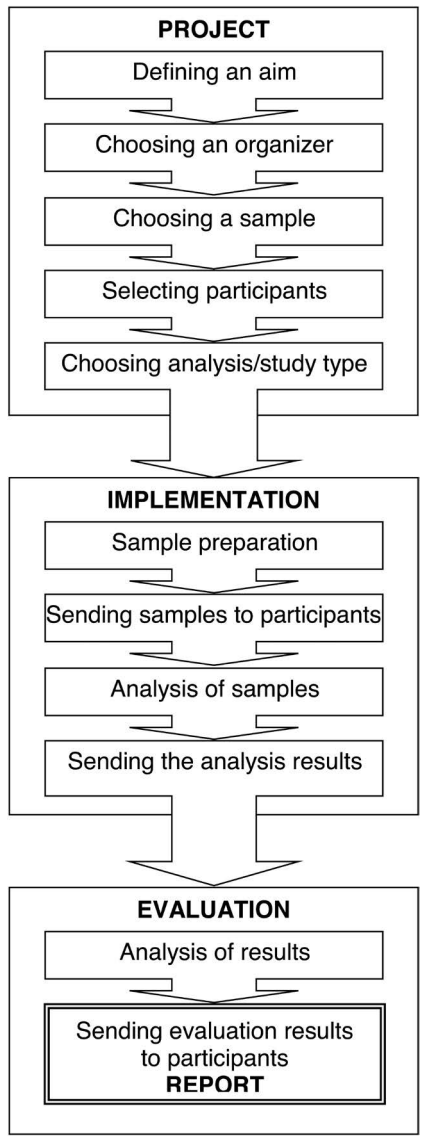


Figure 7.1 A generalized outline for conducting interlaboratory studies [4].

7.3 Classification of Interlaboratory Studies

Interlaboratory studies are organized in order to:

- assess the reliability of measurement results,
- gain experience,
- increase the quality of conducted analytical determinations,
- create possibilities for proving the competence of a given laboratory,
- better understand the applied procedures,
- determine validation parameters.

Laboratories that wish to confirm their competence should participate in at least one program of interlaboratory research. Accredited laboratories are obliged to provide certificates of participation in such a program, both on a national and international scale.

Interlaboratory comparisons may also be classified according to the aim and range of studies. This may include the following:

- method performance study,
- competence study,
- certification study,
- proficiency testing.

Method performance study is an interlaboratory comparison in which all participants act according to the same protocol and use the same test procedures to determine the characteristic features (specified in the protocol) in a batch of identical test samples. The obtained results are applied in estimating the characteristic parameters of the procedure:

- intra – and interlaboratory precision,
- systematic error,
- recovery value,
- internal parameters of quality assurance,
- sensitivity,
- limit of detection,
- applicability limit.

In this type of research, it is necessary to conform to the following requirements:

- the composition of the applied material or sample is usually similar to that of the materials or samples subjected to routine

studies, with regard to the composition of the matrix, analyte concentration and the presence of interferents (the participants of the research are usually informed about the composition of the matrix for the examined samples),
- the number of participants, test samples and determinations as well as other details of the study are presented in the research protocol prepared by the organizer of the study,
- by using the same materials or test samples, it is possible to compare a few procedures; all participating laboratories apply the same set of guidelines for each procedure, and the statistical analysis of the obtained sets of results is conducted separately for each of the procedures.

Competence study is a research in which one or more analyses are carried out by a group of laboratories using one or more homogenous and stable test samples and using a selected or routinely used procedure by each of the laboratories participating in the interlaboratory comparison. The obtained sample results are compared with the results obtained by other laboratories or with a known or determined (guaranteed) reference value. This research may be conducted among laboratories that are accredited or applying for accreditation in order to control the quality of determinations and the proficiency of researchers. In this case, the applied analytical procedure may be a top-down decision or the organizer may limit the choice to a prepared list.

Certification study is a study which assigns a reference value to a given parameter (e.g. analyte concentration, physical property) in a tested material or a given sample, usually with a determined uncertainty. This research is usually carried out by laboratories with a confirmed competence (reference laboratories) to test the material, which is a candidate for the reference material, using a procedure that ensures the estimation of the concentration (or any other parameter) with the smallest error and the lowest uncertainty value.

Proficiency testing is the most frequent type of the interlaboratory research, which is why it is important to pay it a little more attention. These studies are conducted to test the achievements and competence of both the individual analysts using a given analytical procedure or measurement, and a specific analytical procedure.

Proficiency testing may be conducted on the basis of the same material analysis, sample of the material being provided to all the

participants at the same time for a simultaneous study or a round robin-test. In the latter case, some problems with the stability and homogeneity of samples may occur, due to the spread of the studies over a longer time.

Proficiency testing may be conducted as open (public) studies or as a closed study (not public). In the case of closed research, the participants do not know that these are proficiency studies and that the obtained samples are to be analyzed in a routine fashion [5].

Proficiency research is a tremendous challenge for laboratories that need to apply for accreditation based on the presentation of confirmation of their own competence. It is a significant element in achieving and maintaining a suitable quality of results. In proficiency testing, the competence of the participating laboratories is verified based on the determination of results of specified components in distributed samples (materials). Each laboratory is assigned an identification number, under which the participant remains anonymous to the rest of the group.

The choice of test material should be influenced by the maximum degree of similarity of the composition of the samples, usually subjected to analysis with regard to the matrix composition and the level of analyte concentration. Such a material must be tested before it is distributed to the participants, with regard to the mean level of analyte concentration and the homogeneity degree. The obtained results are compared with the previously determined guaranteed (assigned) value.

There are six various ways enabling the determination of the assigned value:

- measurement by a reference laboratory,
- certified value for CRM used as a test material,
- direct comparison of the PT test material with CRM,
- consensus value from expert laboratories,
- formulation value assignment on the basis of proportions used in a solution or other mixture of ingredients with known analyte contents,
- consensus value from participating laboratories.

Sometimes, pilot studies are implemented to select the participants with suitable qualification to participate in the actual proficiency

studies, the so-called key comparisons. After the initial research, all the participants gather to discuss the obtained results. In the case of results distinctly deviating from the assumed range of acceptable results, the participants try to find the causes of the discrepancies. It gives laboratories a chance to improve their competence, correct the hitherto existing mistakes, and improve their performance in the next proficiency test.

With regard to conditions, there are two main types of proficiency studies:

- those examining the competence of the group of laboratories using the results from specifically defined types of analyses,
- those examining the competence of laboratories during the performance of various types of analyses.

Taking into consideration the sample preparation used by the participating laboratories, each of the aforementioned types may be divided into three further categories:

- samples circulate successively from one laboratory to another. In this case, a sample may be taken back to the coordinating laboratory before a test by a subsequent participant, to check if the sample has not changed in an undesirable fashion,
- subsamples randomly selected from a large batch of homogeneous material or test samples are simultaneously distributed to participating laboratories (the most popular type of proficiency testing),
- product or material samples are divided into several parts, and each participant receives one part of each sample (this type is called the split sample study).

There are certain limitations associated with performance and participation in proficiency testing. First of all, proficiency testing is unusually time-consuming. It generally takes a long time before the participants get to know the obtained results. Moreover, the interlaboratory comparisons are retrospective studies, which is why proficiency testing may not affect any decision on quality management. In reality, proficiency testing accounts for only a small percentage of analyses conducted by the laboratories and therefore does not reflect the full picture of routinely performed studies.

7.4 Characteristics and Organization of Interlaboratory Comparisons

As one can see from this current discussion, it is necessary to check the work of individual laboratories because it gives them a chance to estimate the reliability of the analytical results of a given research team. Moreover, a thorough analysis of an analytical process, with the cooperation of a control center, produces a precise localization of sources and causes of errors and hence an improvement in the quality of analytical results. The achievement of these aims requires a painstaking and reliable organization of this research.

Reference materials are a necessary tool to conduct interlaboratory comparisons. Their production and certification is usually very expensive; therefore, the use of certified reference materials (CRM) should be limited to the verification of analytical procedures, and, in the case of comparative methods, it should be limited to the calibration of the control and measuring instruments. Due to economic reasons in interlaboratory comparisons, one may effectively use laboratory reference materials (LRM).

All the reference materials should fulfill basic requirements with regard to similarity, homogeneity and stability over a sufficiently long time. Detailed information on the characteristics, production and implementation of the reference materials is presented in Chapter 6.

7.5 The Presentation of Interlaboratory Comparison Results. Statistical Analysis in Interlaboratory Comparisons

The first stage of interlaboratory research result processing is the graphical presentation of the results [6–9]. To this end, a graph may be constructed where the results are marked from the lowest to the highest, assigning each result a code corresponding to the code number of the laboratory. Diagrams of this type are usually presented in final reports by the organizers of interlaboratory comparisons and proficiency tests. The diagrams make it possible for participants to see how their results relate to the results provided by the other participants. They are also a precious source of information for a potential customer or the accreditation office. On the *X*-axis, laboratory

codes are marked, and/or the applied procedures, and (optionally) the number of performed independent determinations. On the *Y*-axis, the general mean (or assigned value) is marked along with the determined uncertainty value, the individual results obtained by the laboratories and the uncertain values.

Example 7.1

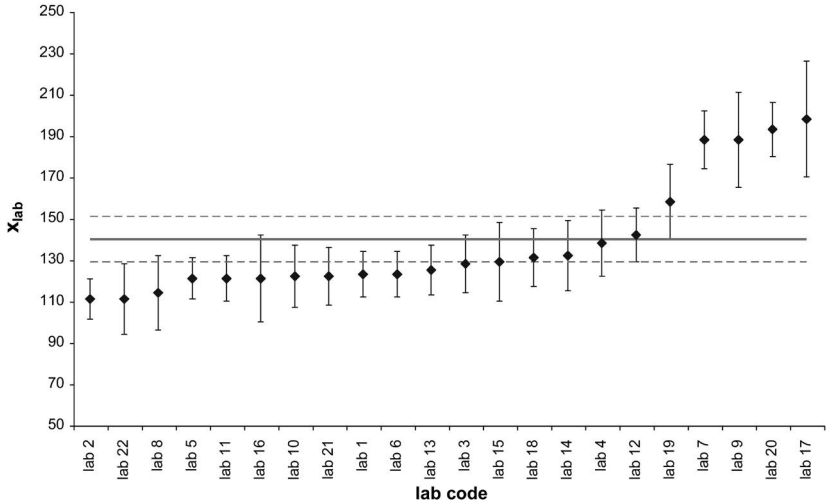
Problem: For a given series of measurement results obtained by various laboratories and a given reference value and its uncertainty, make a diagram showing the distribution of individual determination results.

Data: results:

	DATA	*u*
lab 1	123	11
lab 2	111.0	9.8
lab 3	128	14
lab 4	138	16
lab 5	121	10
lab 6	123	11
lab 7	188	14
lab 8	114	18
lab 9	188	23
lab 10	122	15
lab 11	121	11
lab 12	142	13
lab 13	125	12
lab 14	132	17
lab 15	129	19
lab 16	121	21
lab 17	198	28
lab 18	131	14
lab 19	158	18
lab 20	193	13
lab 21	122	14
lab 22	111	17

SOLUTION:

x_{ref}	**140**
u_{ref}	**11**



Excel file: exampl_7_1.xls

The manner of conducting a statistical analysis of results obtained in interlaboratory comparisons, and the selection of suitable tests and solutions depend on the type of research. Respective documents define the precise manner of conduct for a specified type of research. The ultimate aim of all types of studies is to determine, based on experimentally obtained numerical data, the accuracy (and/or precision) of the measurement procedures. On this basis, one may draw conclusions on the applied procedure and the characteristics of the analyst, compare various procedures and conduct certification of the material or validation of a specified procedure.

The accuracy of a given measurement procedure may be determined by comparing the assumed reference/assigned value with the mean value of results obtained using the said procedure. Depending on the type of measurements and the requirements for the results, one may use the arithmetical mean or median (parameters presented and defined in Chapter 1).

Precision is associated with the conformity of the series of results. In recording, the variability of the results obtained using a given procedure, there are two useful methods of describing precision: repeatability and reproducibility of results obtained using the specified analytical procedures.

At the initial processing of data provided by the participants of interlaboratory comparisons, the distribution type is examined. The

normality of the distribution may be examined using, for example, a *Kolmogorov–Smirnov* test (Section 1.8.18).

The next step in statistical analysis is to eliminate any deviating results. One checks if the occurrence of doubtful or deviating values may be explained by technical errors. A large number of doubtful and/or deviating values (outliers) may suggest a significant discrepancy of the variance values or significant differences in the competence between individual laboratories participating in the project, or lastly may question the suitability of the selected measurement procedure.

Eliminating the outliers is especially crucial in a situation where the material used in the interlaboratory research is a material for which the reference value is determined based on the results of the very research, for example, when it is a certification study, or when the subject of the comparisons is not the reference material.

To this end, one may use the statistical tests of *Cochran* and *Grubbs* [10], or the Hampel test, also called a Huber test [10]. The choice of a suitable test is conditioned by many factors, first of all the number of results. There are many reports in which authors critically examined, analyzed and compared various tests used for outlier rejection.

Example 7.2

Problem: Find outliers in a given series of measurement results obtained by various laboratories, using the Hampel test.

Data: results:

	DATA		DATA
lab 1	123	**lab 12**	142
lab 2	111	**lab 13**	125
lab 3	128	**lab 14**	132
lab 4	138	**lab 15**	129
lab 5	121	**lab 16**	121
lab 6	123	**lab 17**	198
lab 7	188	**lab 18**	131
lab 8	114	**lab 19**	158
lab 9	188	**lab 20**	193
lab 10	122	**lab 21**	122
lab 11	121	**lab 22**	111

SOLUTION:

	$\lvert r_i \rvert$	DATA	OUTLIER OR NOT
lab 1	3.5	123	OK
lab 2	15.5	111	OK
lab 3	1.5	128	OK
lab 4	11.5	138	OK
lab 5	5.5	121	OK
lab 6	3.5	123	OK
lab 7	61.5	188	Outlier!!!
lab 8	12.5	114	OK
lab 9	61.5	188	Outlier!!!
lab 10	4.5	122	OK
lab 11	5.5	121	OK
lab 12	15.5	142	OK
lab 13	1.5	125	OK
lab 14	5.5	132	OK
lab 15	2.5	129	OK
lab 16	5.5	121	OK
lab 17	71.5	198	Outlier!!!
lab 18	4.5	131	OK
lab 19	31.5	158	Outlier!!!
lab 20	66.5	193	Outlier!!!
lab 21	4.5	122	OK
lab 22	15.5	111	OK

SD	**8,5**	**after outlliers rejected**
X_m	**124,4**	

Excel file: exampl_7_2.xls

Example 7.3

Problem: Find outliers in the given sets of measurement results obtained in interlaboratory comparisons. Use the *Cochran* test to examine the intralaboratory variability.

Data: results:

lab 1	12.1	12.6	13.4
lab 2	11.8	12.0	11.4
lab 3	12.8	14.1	13.5
lab 4	11.8	12.1	13.1
lab 5	11.4	10.9	11.0
lab 6	12.6	11.5	13.1
lab 7	13.6	14.1	12.6
lab 8	14.1	12.8	13.7

SOLUTION:

	MEAN	*SD*	*V*
lab 1	12.70	0.66	0.430
lab 2	11.73	0.31	0.093
lab 3	13.47	0.65	0.423
lab 4	12.33	0.68	0.463
lab 5	11.10	0.26	0.070
lab 6	12.40	**0.82**	**0.670**
lab 7	13.43	0.76	0.583
lab 8	13.53	0.67	0.443

n	**3**
p	**8**
C	**0.211**
$C_{0.05}$	0.516
$C_{0.01}$	0.615

Conclusion: Result obtained by laboratory "lab 6" is correct.

Excel file: exampl_7_3.xls

Example 7.4

Problem: Find outliers in the given sets of results obtained in interlaboratory comparisons from Example 7.3. Apply the Grubbs' test for one outlier to examine the interlaboratory variability.

Data: results:

lab 1	12.1	12.6	13.4
lab 2	11.8	12.0	11.4
lab 3	12.8	14.1	13.5
lab 4	11.8	12.1	13.1
lab 5	11.4	10.9	11.0
lab 6	12.6	11.5	13.1
lab 7	13.6	14.1	12.6
lab 8	14.1	12.8	13.7

SOLUTION:

	MEAN
lab 1	12.70
lab 2	11.73
lab 3	13.47
lab 4	12.33
lab 5	**11.10**
lab 6	12.40
lab 7	13.43
lab 8	13.53

n	3
p	8
x_m	12.588
SD	0.881
G_p	1.688
Min/Max	Min
$G_{0.01}$	2.274
$G_{0.05}$	2.126

Conclusion: Result obtained by laboratory "lab 5" is correct.

Excel file: exampl_7_4.xls

To simultaneously determine the standard deviation as the measures of repeatability and reproducibility, one may perform a one-factor (one-dimensional) variance analysis (ANOVA). This analysis serves to verify the hypothesis that the means in the groups are identical against the alternative hypothesis (at least two means are different).

The obtained numerical data are divided into m groups, according to their origin (m – the number of laboratories). When significant differences are found between the values of random errors (statistically significant differences in the variance values), the data are joined into groups for which the variance values are not statistically significantly different, and then the variance analysis is conducted for each group.

An essential condition for conducting a correct interpretation of results for this analysis is the normal distribution of the population from which the samples were taken, with the identical value of the variance V. The essence of the variance analysis is the division of the total variability, that is, the total sum of the squared deviations from

all the measurement from the mean, by the sum of squares describing the variability within groups and the sum of squares describing the variability among groups. Then, one should determine the total intra- and intergroup degrees of freedom and calculate the standard deviation within individual groups and among the groups, the standard deviation being the measure of the respective variances.

The reliability of conclusions depends, to a great extent, on the number of laboratories participating in the research. Below four degrees of freedom, the value of the parameter $t(\alpha, f)$ increases considerably and the precision of the evaluation decreases. It shows that the interlaboratory studies should involve at least five laboratories. The lower influence on the size of the certainty range is exerted by the number of parallel analyses conducted at a given laboratory. The number of parallel determinations that is greater than five occurs only in special cases, or when for some reason one expects deviation of the obtained measurement results from the normal distribution.

Situations in which a single factor completely explains a given phenomenon are rare. A total error, characterizing the results obtained by using an analytical procedure, consists of a few errors which are summed up according to the law of error propagation.

The most often used parameters used to evaluate the obtained results in interlaboratory comparisons is the *Z*-Score parameter. The manner of calculating this parameter has been described in detail in Chapter 1 (Section 1.8.15). The numerical value of the *Z*-Score parameter depends on the number and the type of data available to an analyst:

- when only the mean values obtained from the participating laboratories are known, the assigned (reference) values and the standard deviation sample are calculated according to all the results as the mean value and standard deviations, of course, after rejecting the outliers,

Example 7.5

Problem: In the series of measurement results given in Example 7.1, find which results are satisfactory, which are questionable and which are unsatisfactory. Use the *Z*-Score. Draw a graph with *Z*-Score values for each of the laboratories.

Data: results:

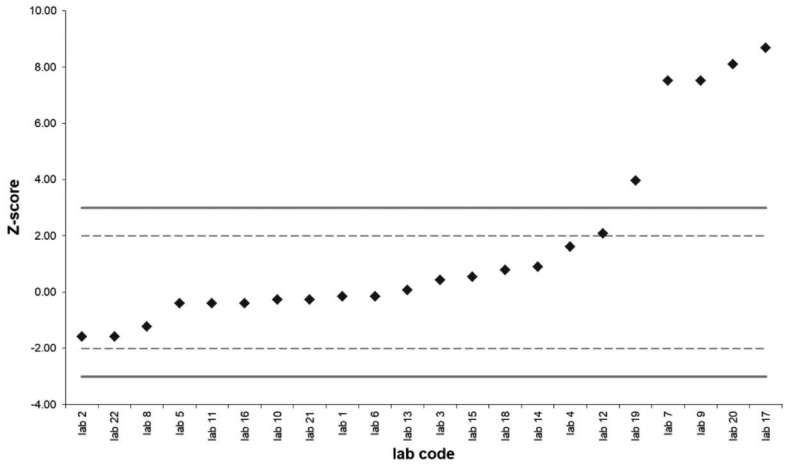
	DATA		DATA
lab 1	123	**lab 12**	142
lab 2	111	**lab 13**	125
lab 3	128	**lab 14**	132
lab 4	138	**lab 15**	129
lab 5	121	**lab 16**	121
lab 6	123	**lab 17**	198
lab 7	188	**lab 18**	131
lab 8	114	**lab 19**	158
lab 9	188	**lab 20**	193
lab 10	122	**lab 21**	122
lab 11	121	**lab 22**	111

SOLUTION:

	Z	CONCLUSION
lab 1	−0.16	Satisfactory
lab 2	−1.58	Satisfactory
lab 3	0.43	Satisfactory
lab 4	1.61	Satisfactory
lab 5	−0.40	Satisfactory
lab 6	−0.16	Satisfactory
lab 7	7.51	Unsatisfactory
lab 8	−1.22	Satisfactory
lab 9	7.51	Unsatisfactory
lab 10	−0.28	Satisfactory
lab 11	−0.40	Satisfactory
lab 12	2.08	Questionable
lab 13	0.08	Satisfactory
lab 14	0.90	Satisfactory
lab 15	0.55	Satisfactory
lab 16	−0.40	Satisfactory
lab 17	8.69	Unsatisfactory
lab 18	0.78	Satisfactory
lab 19	3.97	Unsatisfactory
lab 20	8.10	Unsatisfactory
lab 21	−0.28	Satisfactory
lab 22	−1.58	Satisfactory

x_m	124.4
SD	8.5

Graph:



Excel file: example_7_5.xls

- known mean values obtained by the participating laboratories and known assigned/reference value – the value of standard deviation is calculated according to the total set of measurement results – obviously after rejecting the outliers.

Example 7.6

Problem: In the series of measurement results given in Example 7.1, find for a given reference value which results are satisfactory, which are questionable and which are unsatisfactory. Use the *Z*-Score. Draw a graph with *Z*-Score values for each of the laboratories.

Data: results:

	DATA		DATA
lab 1	123	**lab 12**	142
lab 2	111	**lab 13**	125
lab 3	128	**lab 14**	132
lab 4	138	**lab 15**	129
lab 5	121	**lab 16**	121
lab 6	123	**lab 17**	198
lab 7	188	**lab 18**	131
lab 8	114	**lab 19**	158
lab 9	188	**lab 20**	193
lab 10	122	**lab 21**	122
lab 11	121	**lab 22**	111

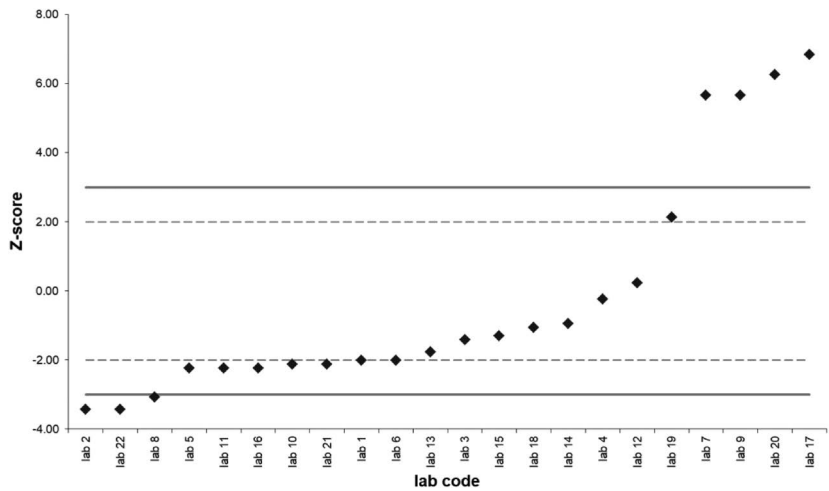
x_{ref}	140

SOLUTION:

SD	8.5

	Z	CONCLUSION
lab 1	−2.01	Questionable
lab 2	−3.42	Unsatisfactory
lab 3	−1.42	Satisfactory
lab 4	−0.24	Satisfactory
lab 5	−2.24	Questionable
lab 6	−2.01	Questionable
lab 7	5.67	Unsatisfactory
lab 8	−3.07	Unsatisfactory
lab 9	5.67	Unsatisfactory
lab 10	−2.13	Questionable
lab 11	−2.24	Questionable
lab 12	0.24	Satisfactory
lab 13	−1.77	Satisfactory
lab 14	−0.94	Satisfactory
lab 15	−1.30	Satisfactory
lab 16	−2.24	Questionable
lab 17	6.85	Unsatisfactory
lab 18	−1.06	Satisfactory
lab 19	2.13	Questionable
lab 20	6.26	Unsatisfactory
lab 21	−2.13	Questionable
lab 22	−3.42	Unsatisfactory

Graph:



Excel file: exampl_7_6.xls

- known mean values obtained by the participating laboratories and known assigned/reference values, and the value of the reference combined uncertainty for a given material,

Example 7.7

Problem: In the series of measurement results given in Example 7.1, find which of the results are satisfactory, questionable or unsatisfactory, for the given reference value and the combined uncertainty reference value. Use the *Z*-Score. Draw a graph with the *Z*-Score values for each of the laboratories.

Data: results:

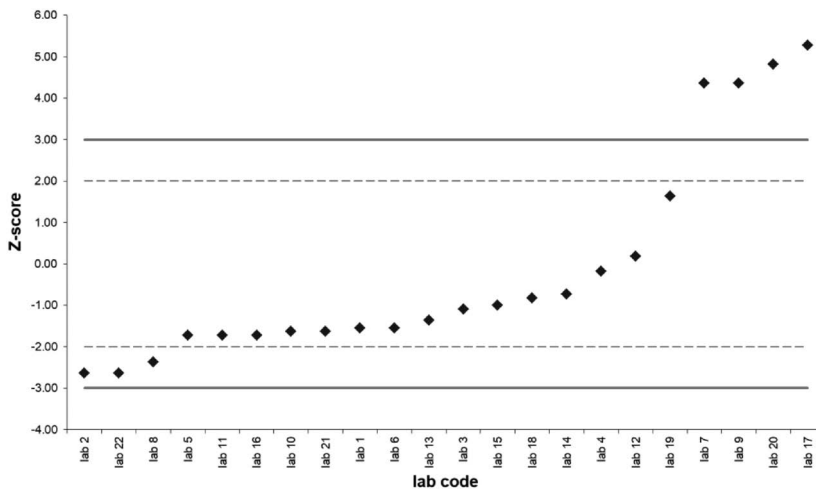
	DATA
lab 1	123
lab 2	111
lab 3	128
lab 4	138
lab 5	121
lab 6	123
lab 7	188
lab 8	114
lab 9	188
lab 10	122
lab 11	121
lab 12	142
lab 13	125
lab 14	132
lab 15	129
lab 16	121
lab 17	198
lab 18	131
lab 19	158
lab 20	193
lab 21	122
lab 22	111

x_{ref}	140
u_{ref}	11

SOLUTION:

	Z	CONCLUSION
lab 1	−1.55	Satisfactory
lab 2	−2.64	Questionable
lab 3	−1.09	Satisfactory
lab 4	−0.18	Satisfactory
lab 5	−1.73	Satisfactory
lab 6	−1.55	Satisfactory
lab 7	4.36	Unsatisfactory
lab 8	−2.36	Questionable
lab 9	4.36	Unsatisfactory
lab 10	−1.64	Satisfactory
lab 11	−1.73	Satisfactory
lab 12	0.18	Satisfactory
lab 13	−1.36	Satisfactory
lab 14	−0.73	Satisfactory
lab 15	−1.00	Satisfactory
lab 16	−1.73	Satisfactory
lab 17	5.27	Unsatisfactory
lab 18	−0.82	Satisfactory
lab 19	1.64	Satisfactory
lab 20	4.82	Unsatisfactory
lab 21	−1.64	Satisfactory
lab 22	−2.64	Questionable

Graph:



Excel file: exampl_7_7.xls

- known mean values obtained in the participating laboratories and known value of the reference combined uncertainty for a given material.

Example 7.8

Problem: In a series of measurement results given in Example 7.1, use *Z*-Score again, taking into consideration the combined uncertainty reference value. Draw a graph with the *Z*-Score values for each of the laboratories.

Data: results:

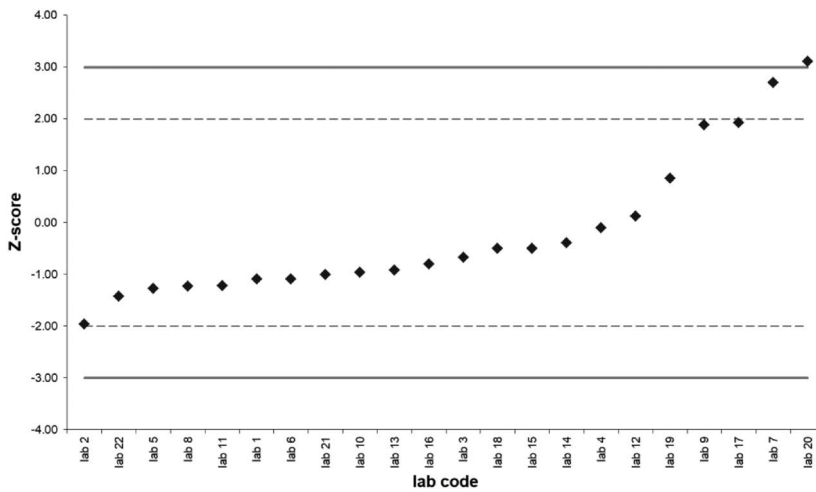
	DATA	*u*
lab 1	123	11
lab 2	111.0	9.8
lab 3	128	14
lab 4	138	16
lab 5	121	10
lab 6	123	11
lab 7	188	14
lab 8	114	18
lab 9	188	23
lab 10	122	15
lab 11	121	11
lab 12	142	13
lab 13	125	12
lab 14	132	17
lab 15	129	19
lab 16	121	21
lab 17	198	28
lab 18	131	14
lab 19	158	18
lab 20	193	13
lab 21	122	14
lab 22	111	17

x_{ref}	140
u_{ref}	11

SOLUTION:

	Z	CONCLUSION
lab 1	−1.09	Satisfactory
lab 2	−1.97	Satisfactory
lab 3	−0.67	Satisfactory
lab 4	−0.10	Satisfactory
lab 5	−1.28	Satisfactory
lab 6	−1.09	Satisfactory
lab 7	2.70	Questionable
lab 8	−1.23	Satisfactory
lab 9	1.88	Satisfactory
lab 10	−0.97	Satisfactory
lab 11	−1.22	Satisfactory
lab 12	0.12	Satisfactory
lab 13	−0.92	Satisfactory
lab 14	−0.40	Satisfactory
lab 15	−0.50	Satisfactory
lab 16	−0.80	Satisfactory
lab 17	1.93	Satisfactory
lab 18	−0.51	Satisfactory
lab 19	0.85	Satisfactory
lab 20	3.11	Unsatisfactory
lab 21	−1.01	Satisfactory
lab 22	−1.43	Satisfactory

Graph:



Excel file: exampl_7_8.xls

Another parameter of the individual examination of the measurement results is the relative error. It is calculated in instances when the participants of a given study use various methods to evaluate the obtained results, and therefore there is no ground to assume a common value of the sample. It is calculated using the formula:

$$\varepsilon = \frac{x_{lab} - x_{ref}}{x_{ref}}[\%] \tag{7.1.}$$

where:

ε – relative error, %,

x_{lab} – the value of the result obtained by a given laboratory,

x_{ref} – reference value.

The evaluation of the obtained results is obvious in this case and depends on the range of analyte concentrations in a given sample. It is assumed that:

- if $|\varepsilon| \leq x$, the evaluation is satisfactory,
- if $|\varepsilon| > x$, the evaluation is not satisfactory,

where:

x – *relative systematic error* (relative deviation), assumed as a limit (permissible).

Example 6.9

Problem: For the data from Example 7.1, calculate the values of the relative errors and make an evaluation for the permissible error value ±20%

Data: results:

	DATA		DATA
lab 1	123	**lab 12**	142
lab 2	111.0	**lab 13**	125
lab 3	128	**lab 14**	132
lab 4	138	**lab 15**	129
lab 5	121	**lab 16**	121
lab 6	123	**lab 17**	198
lab 7	188	**lab 18**	131
lab 8	114	**lab 19**	158
lab 9	188	**lab 20**	193
lab 10	122	**lab 21**	122
lab 11	121	**lab 22**	111

x_{ref}	140
x, %	20.0

SOLUTION:

	ε, %	CONCLUSION
lab 1	−12.1	Satisfactory
lab 2	−20.7	Unsatisfactory
lab 3	−8.6	Satisfactory
lab 4	−1.4	Satisfactory
lab 5	−13.6	Satisfactory
lab 6	−12.1	Satisfactory
lab 7	34.3	Unsatisfactory
lab 8	−18.6	Satisfactory
lab 9	34.3	Unsatisfactory
lab 10	−12.9	Satisfactory
lab 11	−13.6	Satisfactory
lab 12	1.4	Satisfactory
lab 13	−10.7	Satisfactory
lab 14	−5.7	Satisfactory
lab 15	−7.9	Satisfactory
lab 16	−13.6	Satisfactory
lab 17	41.4	Unsatisfactory
lab 18	−6.4	Satisfactory
lab 19	12.9	Satisfactory
lab 20	37.9	Unsatisfactory
lab 21	−12.9	Satisfactory
lab 22	−20.7	Unsatisfactory

Excel file: exampl_7_9.xls

The next parameter of the individual evaluation (for each of the laboratories) of the obtained results is E_n. The method of its determination is described in detail in Chapter 1 (Section 1.8.16).

E_n is a parameter which is decidedly less restrictive than, for example, the standardized Z coefficient because of the inclusion of the uncertainty value. Results that are deemed satisfactory may include values significantly deviating from the mean, but within

the accepted interval, solely attributable to the high value of the extended uncertainty. An opposite situation is possible – a result closer to the mean (compared with another result from a given series) but with the smaller value of extended uncertainty may be considered an outlier.

Example 7.10

Problem: For the data from Example 7.1, apply E_n Score.

Data: results:

	DATA	u
lab 1	123	11
lab 2	111.0	9.8
lab 3	128	14
lab 4	138	16
lab 5	121	10
lab 6	123	11
lab 7	188	14
lab 8	114	18
lab 9	188	23
lab 10	122	15
lab 11	121	11
lab 12	142	13
lab 13	125	12
lab 14	132	17
lab 15	129	19
lab 16	121	21
lab 17	198	28
lab 18	131	14
lab 19	158	18
lab 20	193	13
lab 21	122	14
lab 22	111	17

x_{ref}	140
u_{ref}	11

SOLUTION:

	E_n	CONCLUSION
lab 1	−1.09	Unsatisfactory
lab 2	−1.97	Unsatisfactory
lab 3	−0.67	Satisfactory
lab 4	−0.10	Satisfactory
lab 5	−1.28	Unsatisfactory
lab 6	−1.09	Unsatisfactory
lab 7	2.70	Unsatisfactory
lab 8	−1.23	Unsatisfactory
lab 9	1.88	Unsatisfactory
lab 10	−0.97	Satisfactory
lab 11	−1.22	Unsatisfactory
lab 12	0.12	Satisfactory
lab 13	−0.92	Satisfactory
lab 14	−0.40	Satisfactory
lab 15	−0.50	Satisfactory
lab 16	−0.80	Satisfactory
lab 17	1.93	Unsatisfactory
lab 18	−0.51	Satisfactory
lab 19	0.85	Satisfactory
lab 20	3.11	Unsatisfactory
lab 21	−1.01	Unsatisfactory
lab 22	−1.43	Unsatisfactory

Excel file: exampl_7_10.xls

7.5.1 Comparisons of Results Obtained Using Various Procedures

In this type of comparison, box plots may be used. In the graphical presentation of results, one may examine if the results obtained using various analytical procedures differ among themselves in a statistically significant way. In drawing such a plot, one should divide all the measurement results obtained for a given sample into subsets, each containing results obtained using a specific analytical procedure. Then, for each subset, separate plots are drawn, after which they are all put into one diagram.

Based on data for which the diagrams (plots) are drawn, one calculates the essential values based on the following reasoning:

- ordering the result in a non-decreasing sequence,
- determination of median and quartiles: first (q_1) and third (q_3),
- determination of the interquartile value (IQR), the difference between q_3 and q_1,
- determination of maximum values, whiskers, as 1.5 times the *IQR*.

Based on these calculated values, a diagram (plot) is drawn (separately for a given set of results), in the following manner:

1. on the *OY*-axis, for a given series marked by one point on the *OX*-axis, the values of median and quartiles (q_1 and q_3) are marked – it is a so-called box area representing the middle 50% of the data,
2. on the same plot, whiskers are marked as:
   a. $whisker_{min}$, the minimum value in the set of results, not smaller than the limit equal q_1–1.5·IQR; if the so calculated value is equal to q_1, then the $whisker_{min}$ is not marked on the diagram,
   b. $whisker_{max}$, the maximum value in the set of results, not higher than the limit equal q_3 + 1.5·IQR; if the so calculated value is equal to q_3, then the $whisker_{max}$ is not marked on the diagram.
3. results out of this range (lower than $whisker_{min}$ or higher than $whisker_{max}$) are marked as outliers.

Due to that type of construction of the graph, it is possible to conclude which of the analytical procedures were used more often, and which procedure yields more accurate data.

Example 7.11

Problem: For the data from Example 7.1, construct a boxplot graph.

Data: results:

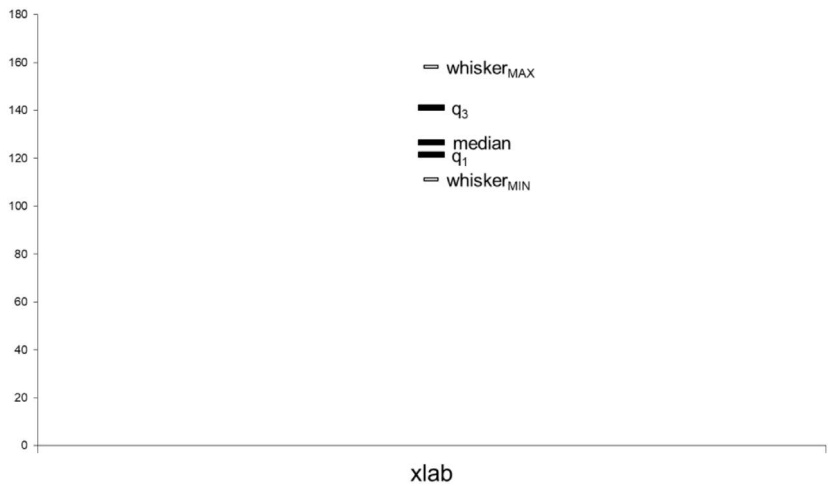
	DATA	u
lab 1	123	11
lab 2	111.0	9.8
lab 3	128	14
lab 4	138	16
lab 5	121	10
lab 6	123	11
lab 7	188	14
lab 8	114	18
lab 9	188	23
lab 10	122	15
lab 11	121	11
lab 12	142	13
lab 13	125	12
lab 14	132	17
lab 15	129	19
lab 16	121	21
lab 17	198	28
lab 18	131	14
lab 19	158	18
lab 20	193	13
lab 21	122	14
lab 22	111	17

SOLUTION:

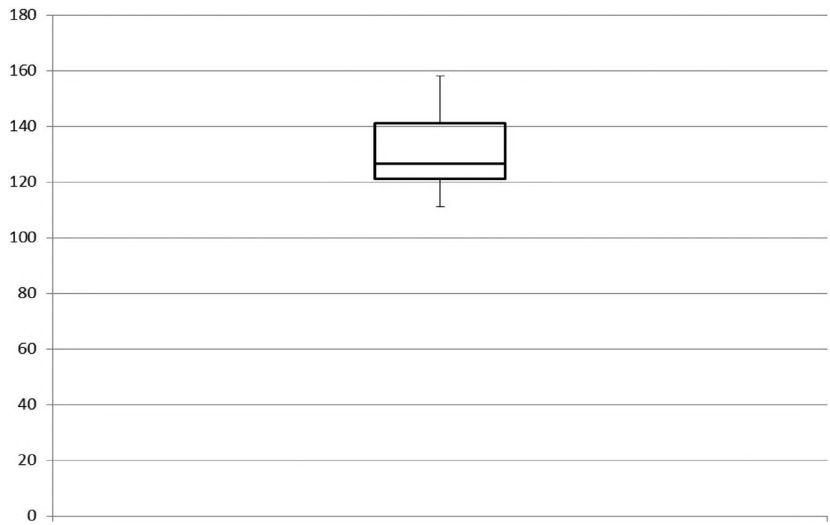
Median – Me	126.5
q_1	121.3
q_3	141.0
IQR	19.8
$1.5 \times$ IQR	29.6
$q_1 - 1.5 \times$ IQR	91.6
$q_3 + 1.5 \times$ IQR	170.6
Min	111
Max	198
$whisker_{min}$	111
$whisker_{max}$	158

	x_{lab}	OUTLIER OR NOT
lab 1	123	OK
lab 2	111	OK
lab 3	128	OK
lab 4	138	OK
lab 5	121	OK
lab 6	123	OK
lab 7	188	Outlier
lab 8	114	OK
lab 9	188	Outlier
lab 10	122	OK
lab 11	121	OK
lab 12	142	OK
lab 13	125	OK
lab 14	132	OK
lab 15	129	OK
lab 16	121	OK
lab 17	198	Outlier
lab 18	131	OK
lab 19	158	OK
lab 20	193	Outlier
lab 21	122	OK
lab 22	111	OK

Graph:



Graph – modified (boxplot):



Excel file: exampl_7_11.xls

7.5.2 Comparison of the Measurement Results Obtained in a Two-Level Study (for Two Samples with Various Analyte Concentrations)

A two-level study is a study where each of the participating laboratories has performed the series of determinations:

- either two series per one sample,
- or determinations for two different samples.

In this case, to determine the presence of systematic errors, a graphical method – also called the *Youden* diagram [8] – may be used. It is an easy and also a very effective method of comparing both intra- and interlaboratory variability. The application of this graph shows which of the participating laboratories achieved comparable results and which laboratory obtained deviating results.

The graph is constructed as follows:

- measurement results for both the obtained series are marked on the *X*- and *Y*-axes,
- solid lines are drawn (both vertical and horizontal) which reflect the values of the main distribution estimators (arithmetic mean or median),

- dotted lines are drawn (also vertical and horizontal) where the distances from the solid lines represent values of the standard deviation from the values of the main distribution estimators (arithmetic mean or median).

The distribution of points on such a constructed diagram is a source of information about what type of error has a dominant impact on the obtained measurement results. When the main cause of the deviations from the mean or median are random errors, the results are distributed in a random manner around the mean (median). If a systematic error is the main cause of differences between the values of the measurement results obtained by the compared laboratories and the mean (median), then the majority of points are in the upper right or bottom left quarter of the graph. It may indicate a positive or negative bias in the analytical procedure applied in a given laboratory.

Example 7.12

Problem: For the two given series of measurement results for two examined samples obtained in the examining laboratories, produce a *Youden* graph.

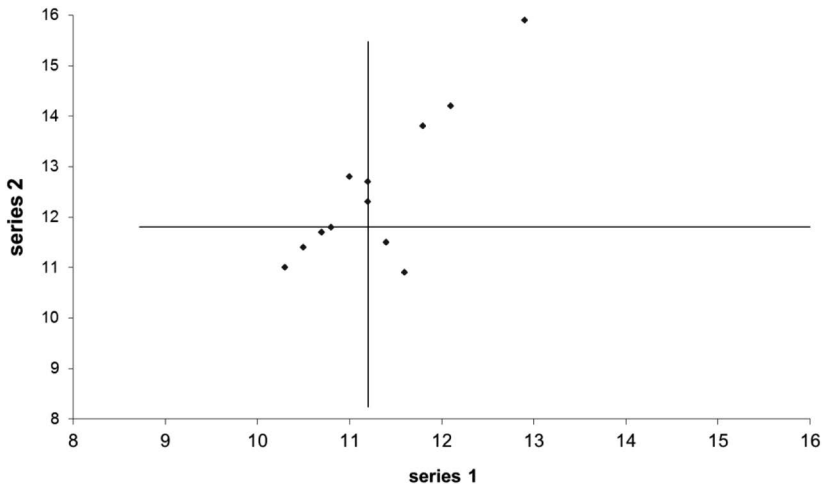
Data: results:

	DATA	
	SERIES 1	SERIES 2
lab 1	11.2	12.3
lab 2	10.8	11.8
lab 3	11	12.8
lab 4	10.7	11.7
lab 5	10.5	11.4
lab 6	10.3	11
lab 7	11.2	12.7
lab 8	11.8	13.8
lab 9	12.1	14.2
lab 10	12.9	15.9
lab 11	10.7	11.7
lab 12	11.6	10.9
lab 13	11.4	11.5

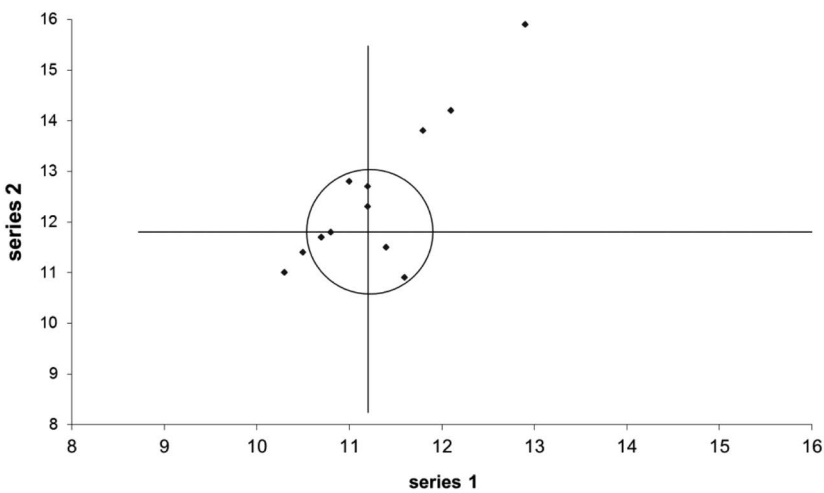
SOLUTION:

	MEDIAN
Series 1	11.2
Series 2	11.8

Graph:



Graph – modified (with 95% limit circle):



Excel file: exampl_7_12.xls

Another, quite common method of graphical presentation of the measurement results obtained by comparing laboratories is the application of Mandel's h and k tests. The application of these tests enables the presentation of the variability of results obtained by using a given analytical procedure and enables an evaluation of a given laboratory. The manner of conducting Mandel's h and k tests is described in Chapter 1 (Section 1.8.17). All laboratories may obtain on different levels of a study (for different analytes or for different concentrations of a single analyte) both positive and negative values of parameter h.

The number of laboratories characterized with positive values of the parameter h should approximate the number of laboratories characterized with negative values. When a laboratory tends to obtain only negative values for h, one may suppose that there is a source of bias for the results obtained by that laboratory.

Similarly, one should pay attention to a situation where all values of parameter h for a given laboratory are characterized with a positive or negative value, and at the same time different from the sign (plus or minus) of the parameter h obtained in other laboratories.

Moreover, when a laboratory yields h values in the extreme range, for example, it achieved an unusually high number of large values of the h parameter, the situation should be adequately explained.

When the graph of the statistical parameter k indicates that a given laboratory deviates from the others due to numerous high values, it shows a smaller repeatability of results obtained by the laboratory compared with the rest of the participants. When the graphs of the h and k connected in groups corresponding to the individual laboratories show that the values of these parameters are close to the lines of critical values, one should pay attention to the problem of systematic errors and the small repeatability of results (great variance value).

Example 7.13

Problem: For a given set of results obtained in the interlaboratory comparison, calculate the values of the Mandel's h test parameter. Draw a graph showing the respective values of the calculated h parameters characterizing the sets of results obtained in individual laboratories.

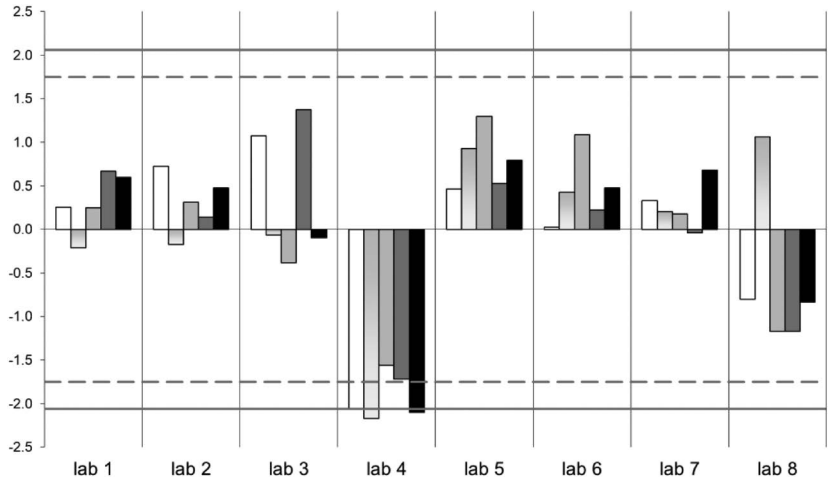
Data: results:

		LEVEL 1	LEVEL 2	LEVEL 3	LEVEL 4	LEVEL 5
lab 1	**1**	4.44	7.21	2.34	14.4	11.3
	2	4.32	7.54	2.01	15.2	11
	3	4.22	7.77	2.15	13.8	12.5
lab 2	**1**	4.98	7.34	2.03	12.7	10.8
	2	4.56	7.77	2.12	13.9	11.5
	3	4.73	7.54	2.44	14.2	11.8
lab 3	**1**	5.11	7.67	1.89	14.8	9.96
	2	5.03	7.83	1.98	16.4	10.4
	3	5.08	7.54	1.78	15.7	10.3
lab 4	**1**	2.22	5.23	1.12	11	6.21
	2	2.11	5.22	1.45	10.6	6.34
	3	2.34	5.01	1.48	10	6.11
lab 5	**1**	4.56	8.67	2.65	14.5	11.8
	2	4.76	9.02	2.73	14.2	12.2
	3	4.23	8.92	2.55	14	12
lab 6	**1**	4.11	8.45	2.22	13.3	11
	2	4.23	8.23	2.86	13.8	11.4
	3	4.02	8.11	2.56	14.1	11.7
lab 7	**1**	4.44	8.11	2.11	13.2	11
	2	4.55	8.02	2.08	13.1	12
	3	4.21	7.88	2.22	13.6	12.3
lab 8	**1**	3.32	8.98	1.56	11.8	8.76
	2	3.35	9.11	1.45	11.3	8.67
	3	3.45	9	1.57	11.2	8.82

SOLUTION:

	LEVEL 1	LEVEL 2	LEVEL 3	LEVEL 4	LEVEL 5
			h		
lab 1	0.2516	−0.2088	0.2441	0.6667	0.5948
lab 2	0.7263	−0.1727	0.3104	0.1414	0.4780
lab 3	1.0759	−0.0643	−0.3822	1.3737	−0.0957
lab 4	−2.0704	−2.1712	−1.5611	−1.7172	−2.0970
lab 5	0.4614	0.9280	1.2977	0.5252	0.7949
lab 6	0.0235	0.4221	1.0840	0.2222	0.4780
lab 7	0.3326	0.2053	0.1778	−0.0404	0.6782
lab 8	−0.8008	1.0615	−1.1706	−1.1717	−0.8312

Graph:



Conclusion: The results obtained by "lab 4" for all the analytes are much lower compared to those obtained by the rest – three of five analytes have exceeded the critical value for the 1% level of significance, which indicates the occurrence of a systematic error source for the results obtained by this laboratory.

Results obtained by the other laboratories are within the permissible range of changes for all the determined analytes.

Excel file: exampl_7_13.xls

Example 7.14

Problem: For a given set of results obtained in an interlaboratory comparison, calculate the values of Mandel's k parameter. Draw a graph showing the respective values of the calculated k parameters characterizing the sets of results obtained in individual laboratories.

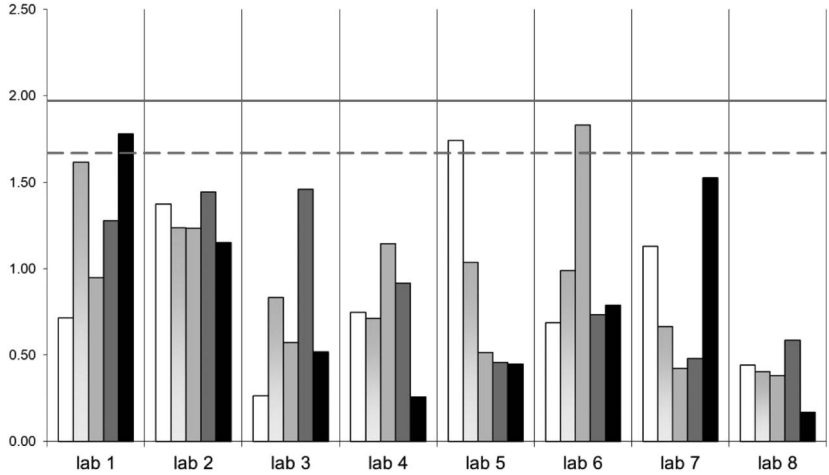
Data: results:

		LEVEL 1	LEVEL 2	LEVEL 3	LEVEL 4	LEVEL 5
lab 1	**1**	4.44	7.21	2.34	14.4	11.3
	2	4.32	7.54	2.01	15.2	11
	3	4.22	7.77	2.15	13.8	12.5
lab 2	**1**	4.98	7.34	2.03	12.7	10.8
	2	4.56	7.77	2.12	13.9	11.5
	3	4.73	7.54	2.44	14.2	11.8
lab 3	**1**	5.11	7.67	1.89	14.8	9.96
	2	5.03	7.83	1.98	16.4	10.4
	3	5.08	7.54	1.78	15.7	10.3
lab 4	**1**	2.22	5.23	1.12	11	6.21
	2	2.11	5.22	1.45	10.6	6.34
	3	2.34	5.01	1.48	10	6.11
lab 5	**1**	4.56	8.67	2.65	14.5	11.8
	2	4.76	9.02	2.73	14.2	12.2
	3	4.23	8.92	2.55	14	12
lab 6	**1**	4.11	8.45	2.22	13.3	11
	2	4.23	8.23	2.86	13.8	11.4
	3	4.02	8.11	2.56	14.1	11.7
lab 7	**1**	4.44	8.11	2.11	13.2	11
	2	4.55	8.02	2.08	13.1	12
	3	4.21	7.88	2.22	13.6	12.3
lab 8	**1**	3.32	8.98	1.56	11.8	8.76
	2	3.35	9.11	1.45	11.3	8.67
	3	3.45	9	1.57	11.2	8.82

SOLUTION:

	LEVEL 1	LEVEL 2	LEVEL 3	LEVEL 4	LEVEL 5
			k		
lab 1	0.7165	1.6163	0.9477	1.2770	1.7792
lab 2	1.3741	1.2355	1.2330	1.4431	1.1503
lab 3	0.2629	0.8341	0.5732	1.4583	0.5170
lab 4	0.7482	0.7133	1.1430	0.9151	0.2585
lab 5	1.7408	1.0352	0.5160	0.4576	0.4483
lab 6	0.6853	0.9901	1.8323	0.7348	0.7872
lab 7	1.1285	0.6655	0.4218	0.4810	1.5258
lab 8	0.4427	0.4019	0.3810	0.5845	0.1692

Graph:



Conclusion: The greatest repeatability for results obtained is achieved by "lab 8".

In the case of individual results ("lab 1", "lab 5" and "lab 6"), the obtained values of repeatability exceed the critical value for the 5% level of significance.

Excel file: exampl_7_14.xls

7.6 Organization of Proficiency Testing – Requirements (ISO 17043)

ISO 17043 [1] is an international standard that specifies general competency requirements for proficiency testing (PT) providers, who must ensure that their operations comply with specific quality and competency standards to meet the requirements of the standard. The key areas of ISO 17043 are:

a. **impartiality and confidentiality:**
   - the PT provider must act objectively, avoiding conflicts of interest that could affect the results of PTs,
   - the PT must protect the confidentiality of all information relating to participants, including the results of individual laboratories,

b. **quality management system:**
   - the PT provider must implement and maintain a quality management system that is properly documented;

this documentation should include policies, procedures, instructions and other documents necessary for the proper functioning of the interlaboratory comparison program,
- the PT provider must identify and manage the risks associated with the implementation of PT,

c. **competencies and qualifications:**
- the PT provider must have the appropriate technical competence and resources to plan, organize and conduct proficiency tests,
- the PT provider must provide regular training for staff in order to maintain and develop their competences; regular assessment of staff competence is necessary to ensure that staff have the appropriate skills to perform their tasks,

d. **technical resources:**
- the PT provider must have the appropriate apparatus, equipment and infrastructure necessary to conduct proficiency tests,
- the equipment used in the tests must be regularly inspected, calibrated and maintained in good condition,

e. **design, planning and development of PTs:**
- the PT program must be carefully planned, taking into account clearly defined PT objectives, the scope of the tests, participants, performance evaluation criteria and timetable,
- the samples used for PT must be adequately prepared, and their stability and homogeneity must be ensured,
- the testing and analytical methods used must be validated and appropriate for the intended purpose of the PT,
- participants should be given clear instructions on the procedures to be followed during the study,
- the PT provider must analyze and compare the results obtained by the participants, as well as evaluate them according to pre-established criteria, and the results of the proficiency test must be clearly presented in the reports that are provided to the participants,
- these reports should include an interpretation of the results and possible recommendations.

Only by carrying out inter-laboratory comparisons in a reliable, objective manner and in accordance with international standards can the competence of the participating laboratories be reliably assessed.

7.7 Conclusions

The ultimate and most reliable manner of estimation of the quality of measurement results obtained by a given laboratory is the comparison of their results with those obtained in other laboratories. Bearing this in mind, laboratories for many years have participated in various inter-laboratory comparisons, both on a national and international scale.

A major task in interlaboratory comparisons is the help offered to a laboratory in detecting all types of irregularities during a given analytical procedure that may affect the reliability of the obtained results. In other words, it is a system of mutual aid where a participant obtains information whether and how they should modify the applied measurement procedure to increase the reliability of the obtained results.

High marks/grades obtained in interlaboratory proficiency studies indicate a high quality of analyses performed by the participating laboratory. The test of the interlaboratory proficiency is used to estimate the reliability of determination results and is the basis for the validation of analytical procedures according to EN 17025, and enables issuance of opinions on organizational procedures. It is hence obvious that laboratories that do not participate in these comparisons are deemed unreliable.

However, while interpreting the results of the interlaboratory studies, one should remember that:

- participation in interlaboratory studies must not serve as a substitute for routine intralaboratory control of the results' quality,
- the results of the interlaboratory studies enable detection and definition of current problems in a given laboratory, and not those that may occur,
- a successful outcome in interlaboratory studies obtained during the determination of a given analyte or a group of analytes may not be automatically related to another analyte or group of analytes; the same applies to an analytical method.

To sum up, the major task of interlaboratory studies is to obtain an explicit answer to the question: "Are the measurement results obtained in a given laboratory as good as we think they are?" (http://www.HN-Proficiency.com).

References

1. ISO/IEC 17043:2023 Conformity assessment – General requirements for the competence of proficiency testing providers.
2. Thompson M., and Ellison S.L.R., Fitness for purpose – the integrating theme of the revised harmonized protocol for proficiency testing in analytical chemistry laboratories, *Accred. Qual. Assur.*, 11, 373–378, 2006.
3. Juniper I.R., Quality issues in proficiency testing, *Accred. Qual. Assur.*, 4, 336–341, 1999.
4. Konieczka P., The role of and place of method validation in the quality assurance and quality control (QA/QC) system, *Crit. Rev. Anal. Chem.*, 37, 173–190, 2007.
5. Analytical Methods Committee, Proficiency Testing of Analytical Laboratories: Organization and statistical assessment, *Analyst*, 117, 97–104, 1992.
6. Vander Heyden Y., and Smeyers-Verbeke J., Set-up and evaluation of interlaboratory studies, *J. Chromatogr. A.*, 1158, 158–167, 2007.
7. Tholen D.W., Statistical treatment of proficiency testing data, *Accred. Qual. Assur.*, 3, 362–366, 1998.
8. Davies P.L., Statistical evaluation of interlaboratory tests, *Fresenius Z. Anal. Chem.*, 331, 513–519, 1988.
9. Eurachem Guide on Selection, *Use and Interpretation of PT Schemes*, 3rd edition, 2021, https://www.eurachem.org/images/stories/Guides/pdf/EPT_2021_P3_EN.pdf (access date 26.06.2024).
10. Linsinger T.P.J., Kandel W., Krska R., and Grasserbauer M., The influence of different evaluation techniques on the results of interlaboratory comparisons, *Accred. Qual. Assur.*, 3, 322–327, 1998.

8
Calibration

8.1 Introduction

Every analytical procedure includes a calibration step. It is particularly important in the case of indirect (relative) measurements, that is, those where the signal from the instrument is a function (very often unknown) of the quantity being measured.

Calibration is defined as "marking, correcting or measuring, scaling" [1]. Calibration is also referred to as "a model that attempts to predict the value of an independent variable when only the dependent variable is known" [2]. In general, the term "analytical calibration" is understood as a process consisting of mapping the actual (true, theoretical) dependence of the analytical signal on the concentration (content) of the analyte onto the empirical form (i.e. the so-called calibration table) and then using this table to determine the concentration (content) of the analyte in the tested sample (i.e. to obtain the so-called analytical result).

Calibration can either be a step in an analytical procedure or just to check the class of the instrument used. With this in mind, we can speak of:

- calibration of an instrument operating on the principle of indirect measurement; in this case, the calibration step is used to assign a value to the signal measured for the test sample on the basis of the value measured for the standard(s),
- verification of the instrument class to determine the linearity of the measuring instrument, verification of the dependence of its readings on changes in the parameters of the standards used, determination of the values of the limits of detection and/or quantification, verification of the "zero" point of the instrument; this type of calibration can be used not only for instruments operating on the principle of indirect measurement, but also for measuring instruments whose principle of

operation is based on direct measurement (e.g. measurement of mass, electrical charge).

8.2 Types of Calibration

IUPAC [3] distinguishes between two different types to calibration:

1. Qualitative calibration (identification and qualitative analysis),
2. Quantitative calibration (quantification of analytes).

Qualitative calibration or identification of analytes is based upon the developed model of calibration of analytical parameters, which characterizes groups of chemical compounds. The models used most often are those in which the identification of analytes is carried out through the following [4]:

1. assignment of a given analyte to a given detector signal on the basis of retention parameters – for example, liquid chromatography (LC), gas chromatography (GC),
2. calibration of a detector against a reference standard (with a known value on the signal scale) and, on this basis, the assignment of values to analytes – for example, nuclear magnetic resonance (NMR),
3. comparison of selected signals of a reference standard (e.g. from the library of spectra) to the signals characteristic of an analyte – for example, mass spectrometry (MS).

   Calibration of a measuring instrument (detector, analyzer, monitor) is generally not a simple task. The method of calibration depends on a number of factors (Kalivas and Sutter 1991):
   - type of instrument,
   - number of samples – analysis time,
   - the possibility of preparing standard samples in a wide range of analyte concentrations (to check the whole range of the instrument),
   - required precision of the measurement result,
   - the required uncertainty of the measurement result,
   - the composition of the sample matrix,
   - the possibility of changing the composition of the sample during the analytical process.

Taking into account the way in which the sample and the standard are treated in the analytical process, a distinction can be made between:

- external calibration – in this case, the standard sample and the tested sample are subjected to the measurement procedure separately; therefore, separate, independent measurements are carried out for each type of sample,
- internal calibration – in this case, the standard is added to the test sample prior to the analytical procedure, and the determination is carried out in a common analytical procedure; in this case, the calibration step minimizes the possible influence of the matrix composition on the measurement result, since it is assumed that the matrix composition has the same effect on the analyte present in the test sample as on the analyte added in the standard.

8.3 Calibration Techniques

The calibration step includes the following unit operations:

- preparation of the standard solution(s),
- carrying out the measurements for the prepared solution(s),
- determination of the relationship between the measured signal value(s) (dependent variable) and the known concentration/content value(s) (independent variable) in the prepared standards.

Taking into account the number of standard solutions prepared, their type and how the calibration relationship is determined, a number of calibration techniques can be distinguished.

8.3.1 Single Standard Technique

In this case, two measurements are carried out:

1. for the standard mixture,
2. for the sample.

The analyte content in the sample is calculated according to the formula:

$$C_x = C_{st} \times \frac{S_x}{S_{st}} \tag{8.1}$$

where:

C_x – analyte content in the sample,
C_{st} – analyte content in the standard,
S_x – measuring device signal for the sample,
S_{st} – the signal of the measuring device for the standard.

The less the analyte content in the sample differs from the analyte content in the standard sample, the more accurate the final result. The narrower the concentration range (a small difference in the concentration levels of the analyte), the more it is possible to approximate even the non-linear relationship between the output signal and the analyte content with a straight line segment. It should be emphasized that this type of calibration is an extrapolation.

Bearing this in mind, it is important to remember that the risk of error is very high when using this type of calibration. It is assumed that the possible noise level is the same in both samples analyzed – the standard and the test sample – which is often not a correct assumption. The possibility of error increases significantly as the difference between the signals received for the standard and the test sample increases. This calibration method is an example of external calibration.

Example 8.1

Problem: On the basis of the measurement results (signals) obtained for the standard sample and the test sample, calculate the concentration of the analyte in the test sample. Use the single standard technique in the calculation.

Data:

S_x	1 456
S_{st}	1 257
C_{st} mg/dm^3	15.2

SOLUTION:

Using the relation:

$$C_x = C_{st} \times \frac{S_x}{S_{st}}$$

the concentration of the analyte in the test sample has been calculated.

Conclusion: The calculated analyte concentration in the test sample was C_x = 17.6 mg/dm^3.

Excel file: exampl_8_1.xls

8.3.2 Bracketing Solutions Technique

In this case, it is necessary to carry out three measurements:

1. for the sample,
2. on the sample of the standard solution in which the content of the analyte is higher than the content of the analyte in the tested sample,
3. on the sample of the standard solution in which the content of the analyte is lower than the content of the analyte in the tested sample.

The analyte content in the sample is calculated according to the one of the formulas:

$$C_x = C_{st\_H} - \frac{\left(C_{st\_H} - C_{st\_L}\right) \times \left(S_{st\_H} - S_x\right)}{S_{st\_H} - S_{st\_L}} \tag{8.2}$$

$$C_x = C_{st\_L} + \frac{\left(C_{st\_H} - C_{st\_L}\right) \times \left(S_x - S_{st\_L}\right)}{S_{st\_H} - S_{st\_L}} \tag{8.3}$$

where:

C_x – analyte content in the sample,

C_{st_H} – analyte content in the standard in which the content of the analyte is higher than the content of the analyte in the tested sample,

C_{st_L} – analyte content in the standard in which the content of the analyte is lower than the content of the analyte in the tested sample,

S_x – measuring device signal for the sample,
S_{st_H} – the signal of the measuring device for the standard in which the content of the analyte is higher than the content of the analyte in the tested sample,
S_{st_L} – the signal of the measuring device for the standard in which the content of the analyte is lower than the content of the analyte in the tested sample.

The smaller the difference between the analyte concentrations in the standard samples, the more accurate the final result. This is a fast method of calibration, particularly recommended for unstable measurements, so it is a very common practice to perform measurements in the following sequence: $st\_L \rightarrow x \rightarrow st\_H \rightarrow\rightarrow x \rightarrow st\_L$. In the case of small differences in analyte concentrations in the standards used, this calibration method can be used even in the case of a non-linear signal concentration dependence. This calibration method is an example of external calibration.

Example 8.2

Problem: On the basis of the measurement results (signals) obtained for the two standard samples and the test sample, calculate the concentration of the analyte in the test sample. Use the bracketing solution technique in the calculation.

Data:

S_x	1456
S_{st_L}	1257
S_{st_H}	1766
C_{st_L} mg/dm^3	15.2
C_{st_H} mg/dm^3	21.5

SOLUTION:

Using the relation:

$$C_x = C_{st\_H} - \frac{\left(C_{st\_H} - C_{st\_L}\right) \times \left(S_{st\_H} - S_x\right)}{S_{st\_H} - S_{st\_L}}$$

the concentration of the analyte in the test sample has been calculated.

Conclusion: The calculated analyte concentration in the test sample was C_x = 17.7 mg/dm^3.

Excel file: exampl_8_2.xls

8.3.3 Calibration Curve Technique

In this instance, a series of standard solutions are employed. It is advised that the standard solutions against which the calibration curve is established meet the following criteria:

- the number of solutions utilized should be a minimum of five (recommendations range from five to seven),
- the solutions should encompass the anticipated concentration/content range of the analyte in the test sample(s),
- the range should encompass no more than three decades of concentrations/contents values,
- the concentration/content range should be distributed evenly.

To establish the functional relationship between the measured signals and the analyte concentrations/concentrations in the standard solutions, a linear regression method is most commonly used, the principles of which, together with the relevant formulae, are described in Subsection 1.9.

Example 8.3

Problem: On the basis of the measurement results (signals) obtained for the seven standard samples and the test sample, calculate the concentration of the analyte in the test sample. Use the calibration curve technique in the calculation.

Data:

C_{st_1} mg/dm^3	3.2	S_{st_1}	213
C_{st_2} mg/dm^3	9.5	S_{st_2}	748
C_{st_3} mg/dm^3	15.2	S_{st_3}	1257
C_{st_4} mg/dm^3	21.5	S_{st_4}	1766
C_{st_5} mg/dm^3	27.1	S_{st_5}	2267
C_{st_6} mg/dm^3	33.2	S_{st_6}	3011
C_{st_7} mg/dm^3	40.1	S_{st_7}	3567
		S_x	1456

SOLUTION:

Using the linear regression method, the slope and intercept values were calculated.

$$S = b \times C + a$$

the slope b = 91.8
the intercept a = −131

The concentration of the analyte in the test sample has been calculated according to the following formula:

$$C_x = \frac{S_x - a}{b}$$

Conclusion: The calculated analyte concentration in the test sample was C_x = 17.3 mg/dm^3.

Excel file: exampl_8_3.xls

Example 8.4

Problem: A comparison of the results obtained for the different calibration techniques is required, as illustrated in Examples 8.1– 8.3. The conclusions that can be drawn from the differences in the results will then be presented.

Data:

CALIBRATION TECHNIQUE	C_x mg/dm^3
Single standard (Example 8.1)	17.6
Bracketing solutions (Example 8.2)	17.7
Calibration curve (Example 8.3)	17.3

Conclusion: The results obtained with the different calibration techniques do not differ significantly.

This is due to the following reasons:

1. in the case of the single standard technique, the signals for the standard and the test sample did not differ significantly,
2. for the limiting solution technique, the difference between the signals for the standards was small,
3. the correlation coefficient in the case of the standard curve technique was very high (0.999) indicating a very good

matching of the points for the standard solution samples; the two calibration points (st_3 and st_4) are the solutions used in the bracketing solutions technique.

Example 8.5

Problem: On the basis of the measurement results (signals) obtained for the standard sample and the test sample, calculate the concentration of the analyte in the test sample. Use the single standard technique in the calculation.

Data:

S_x	1456
S_{st}	452
C_{st} mg/dm^3	15.2

SOLUTION:

Using the relation:

$$C_x = C_{st} \times \frac{S_x}{S_{st}}$$

the concentration of the analyte in the test sample has been calculated.

Conclusion: The calculated analyte concentration in the test sample was C_x = 49.0 mg/dm^3.

Excel file: exampl_8_5.xls

Example 8.6

Problem: On the basis of the measurement results (signals) obtained for the two standard samples and the test sample, calculate the concentration of the analyte in the test sample. Use the bracketing solution technique in the calculation.

Data:

S_x	1456
S_{st_L}	452
S_{st_H}	1766
C_{st_L} mg/dm^3	15.2
C_{st_H} mg/dm^3	21.5

SOLUTION:

Using the relation:

$$C_x = C_{st\_H} - \frac{\left(C_{st\_H} - C_{st\_L}\right) \times \left(S_{st\_H} - S_x\right)}{S_{st\_H} - S_{st\_L}}$$

the concentration of the analyte in the test sample has been calculated.

Conclusion: The calculated analyte concentration in the test sample was C_x = 20.0 mg/dm^3.

Excel file: exampl_8_6.xls

Example 8.7

Problem: On the basis of the measurement results (signals) obtained for the five standard samples and the test sample, calculate the concentration of the analyte in the test sample. Use the calibration curve technique in the calculation.

Data:

C_{st_1} mg/dm^3	15.2	S_{st_1}	452
C_{st_2} mg/dm^3	21.5	S_{st_2}	1766
C_{st_3} mg/dm^3	27.1	S_{st_3}	3233
C_{st_4} mg/dm^3	33.2	S_{st_4}	4127
C_{st_5} mg/dm^3	40.1	S_{st_5}	5623
		S_x	1456

SOLUTION:

Using the linear regression method, the slope and intercept values were calculated.

$$S = b \times C + a$$

the slope b = 206.4
the intercept a = –2618

The concentration of the analyte in the test sample has been calculated according to the following formula:

$$C_x = \frac{S_x - a}{b}$$

Conclusion: The calculated analyte concentration in the test sample was C_x = 19.7 mg/dm^3.

Excel file: exampl_8_7.xls

Example 8.8

Problem: A comparison of the results obtained for the different calibration techniques is required, as illustrated in Examples 8.5– 8.7. The conclusions that can be drawn from the differences in the results will then be presented.

Data:

CALIBRATION TECHNIQUE	C_x mg/dm^3
Single standard (Example 8.5)	49.0
Bracketing solutions (Example 8.6)	20.0
Calibration curve (Example 8.7)	19.7

Conclusion: The results obtained using the single standard technique differ very significantly from the results obtained using the other calibration techniques. The reason for this is the significant difference in signals for the standard and the test sample.

No such significant difference was found for the results obtained using the standard curve and the bracketing solution technique. In the case of the calibration curve technique, a high correlation coefficient value (0.997) was obtained, indicating a very good matching of points for the standard solution samples; two calibration points (st_1 and st_2) are the solutions used in the bracketing solutions technique.

8.3.4 Standard Addition Technique

The measurement is conducted on the sample (or standard) itself and then on the sample with the addition (or loss) of the standard (or vice versa). The linear regression method is used to calculate the result for the test sample. However, in this case, the values of the independent variable (concentration/concentration) are given in terms of the amount of standard added (subtracted). Therefore, the signal value for the test sample is assigned a value of zero (0) on the 0X-axis.

It should be noted, however, that in this case the linear regression is only applied to two measurement points, which may be a source of additional error in the determination of the analyte concentration/concentration in the sample.

The quantity of added standard must be explicitly delineated in order to ensure that:

1. the increase in signal resulting from the addition of the standard must be at least 50% of the signal for the analyte in the sample before the addition, but no greater than 150% of this value,
2. the quantity of the added standard (in mass or volume) must not alter the composition of the test sample matrix,
3. it is essential that the analyte present in the standard is bound to the sample matrix in a manner that is identical to the analyte present in the sample; it is therefore necessary to allow a period of time to elapse after the addition of the standard, in order to establish an equilibrium state between the analyte and the matrix.

The advantage of this method is that the matrix has a minimal impact on the measurement result, as both samples (real and with the addition of a standard) are determined in a matrix with a very similar composition [5–7]. An alternative approach is to employ a subtraction procedure. To illustrate, an agent that reacts with an analyte is introduced to the sample, resulting in the formation of a new compound that has no impact on the detector signal. The quantity of the agent additive is known; therefore, the loss of the analyte can be determined.

An alternative approach to the standard addition technique is the sample dilution or enrichment technique. In this instance, the measurement is conducted on the actual sample and then on the sample that has undergone a dilution (or enrichment) process. In this instance, an additional measurement should be conducted for the pattern due to the lack of knowledge regarding the relationship between the variables. It should also be noted that the added amount of standard (which is small enough) does not significantly affect the composition of the sample matrix. The advantage of this technique is that the modification can result in the concentration of the analyte in the sample being tested at the level found in the standard sample, which improves the accuracy of the determination.

Example 8.9

Problem: A quantity of 100 cm^3 of the test sample was subjected to the addition of 1.3 cm^3 of a 5000 mg/dm^3 standard solution. Utilizing the measured signal values for the test sample and the sample with

standard addition, the analyte content of the test sample was calculated using the single standard addition technique.

Data:

S_x	53.23
S_{x+st}	110.10
C_{st} mg/dm^3	5000
V_x cm^3	100
V_{st} cm^3	1.3

SOLUTION:

Prepare a graph of function S = f ($V_{st\ (added)}$).
For the data obtained:

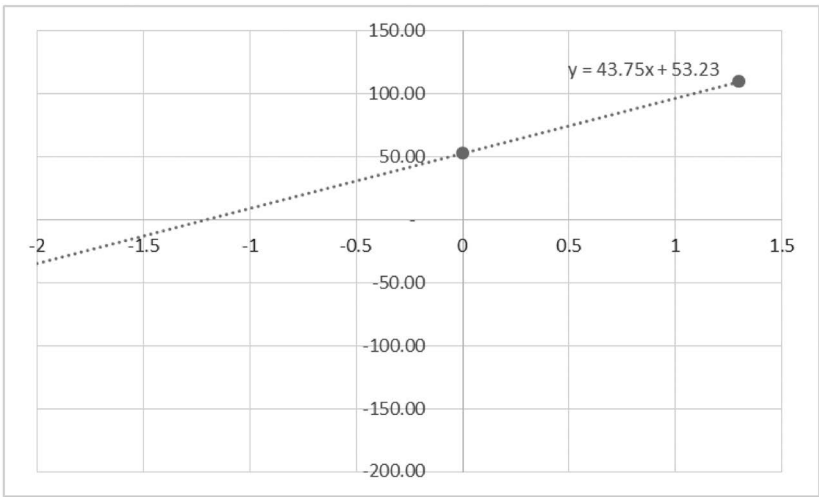
S	$V_{st\ (added)}$ cm^3
53.23	0
110.10	1.3

Using the linear regression method, the slope and intercept values were calculated.

$$S = b \times V_{st(added)} + a$$

the slope b = 43.7
the intercept a = 53.2

Graph:



The concentration of the analyte in the test sample has been calculated according to the following formula:

$$C_x = \frac{a}{b} \times \frac{C_{st}}{V_x}$$

Conclusion: The calculated analyte concentration in the test sample was C_x = 60.8 mg/dm^3.

Excel file: exampl_8_9.xls

8.3.5 Multiple Standard Addition Technique

In order to enhance precision, the standard addition technique employs a variant that entails the multiple standard addition into sample.

It is essential to adhere to the requisite conditions for the application of this technique, as is the case with the standard addition technique.

At the stage of calculation, the method of linear regression is employed to ascertain the relationship between the signal and the amount (in mass or volume) of the added standard.

Example 8.10

Problem: A volume of 1.3 cm^3 of a 5000 mg/dm^3 standard solution was added to 100 cm^3 of the test sample. This process was repeated five times. After each addition of the standard solution, the signal was measured. The signal values for the test sample and samples with standard addition were then measured and used to calculate the analyte content of the test sample using the multiple standard addition technique.

Data:

S_x	53.23
C_{st} mg/dm^3	5000
V_x cm^3	100
V_{st} cm^3	1.3
S_{x+st1}	110.1
S_{x+st1}	154.2
S_{x+st1}	221.3
S_{x+st1}	276.8
S_{x+st1}	331.5

SOLUTION:

Prepare a graph of function S = f ($V_{st\ (added)}$).
For the data obtained:

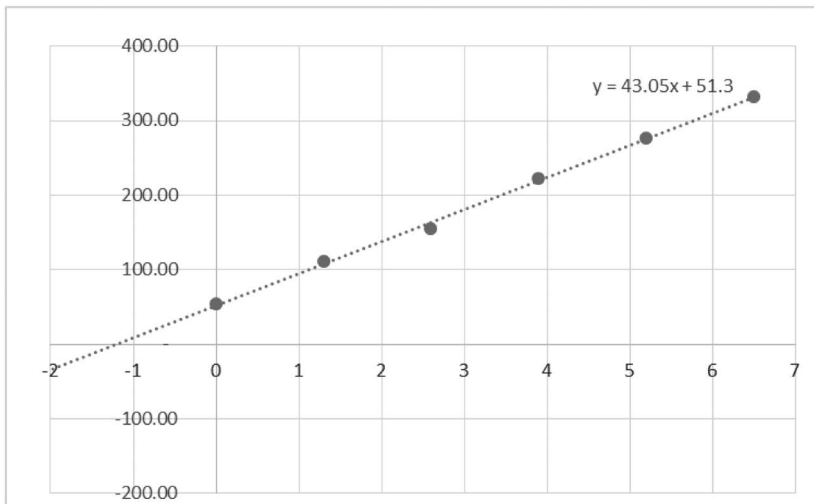
S	$V_{st\ (added)}$ cm^3
53.23	0
110.1	1.3
154.2	2.6
221.3	3.9
276.8	5.2
331.5	6.5

Using the linear regression method, the slope and intercept values were calculated.

$$S = b \times V_{st(added)} + a$$

the slope b = 43.05
the intercept a = 51.3

Graph:



The concentration of the analyte in the test sample has been calculated according to the follwing formula:

$$C_x = \frac{a}{b} \times \frac{C_{st}}{V_x}$$

Conclusion: The calculated analyte concentration in the test sample was C_x = 59.6 mg/dm^3.

Excel file: exampl_8_10.xls

8.3.6 Internal Standard Technique

In this technique, a compound that is not an analyte is employed as a standard. The internal standard is then introduced to the sample to be tested, and the concentration of the analyte is determined by calculating the ratio of the signals for the analyte and the internal standard.

This technique is only applicable to analytical techniques that involve grinding, which encompasses all chromatographic techniques.

In order for a compound to be used as an internal standard, it must meet the following criteria:

1. it must not be present in the sample being tested,
2. it must have similar physical and chemical properties to the analyte,
3. the added amount of internal standard should generate a signal at the level of the signal obtained for the analyte,
4. the added amount of internal standard should not change the composition of the matrix of the test sample,
5. the added amount of internal standard should be measured with a reasonable accuracy.

Example 8.11

Problem: During the calibration stage, an internal standard technique was used. For this purpose, five standard solutions were added such quantities of internal standard at a concentration of 30 mg/dm^3. The same amount of internal standard was added to the test sample. Based on the measurement results obtained for the standard solutions and the test sample, calculate the concentration of the analyte in the test sample.

Data:

C_{st_1} mg/dm³	15.2	S_{st1}	452	S_{IST_1}	3120
C_{st_2} mg/dm³	21.5	S_{st2}	1766	S_{IST_2}	3234
C_{st_3} mg/dm³	27.1	S_{st3}	3233	S_{IST_3}	3167
C_{st_4} mg/dm³	33.2	S_{st4}	4127	S_{IST_4}	3222
C_{st_5} mg/dm³	40.1	S_{st5}	5623	S_{IST_5}	3098
		S_x	1456	S_{IST_x}	3145
C_{IST} mg/dm³	30.0				

SOLUTION:

Calculate the ratio of analyte to IST signals (S_{st_n}/S_{IST_n}) for each of the standard solutions.

Calculate regression parameters of function $S_{st\_n}/S_{IST\_n} = f(C_{st\_n})$.

For the data obtained:

S_{st_n}/S_{IST_n}	C_{st_n} mg/dm³
0.145	15.2
0.546	21.5
1.021	27.1
1.281	33.2
1.815	40.1

Using the linear regression method, the slope and intercept values were calculated.

$$S_{st\_n}/S_{IST} = b \times C_{st\_n} + a$$

the slope $b = 0.0663$

the intercept $a = -0.855$

The concentration of the analyte in the test sample has been calculated according to the following formula:

$$C_x = \frac{\frac{S_x}{S_{IST\_x}} - a}{b}$$

Conclusion: The calculated analyte concentration in the test sample was $C_x = 19.9$ mg/dm³.

Excel file: exampl_8_11.xls

Example 8.12

Problem: In the calibration stage, the internal standard technique was used. For this purpose, five standard solutions were prepared with the addition of a known amount of internal standard.

A known amount of internal standard was also added to the test sample.

Based on the measurement results obtained for the standard solutions and the test sample, calculate the concentration of the analyte in the test sample.

Data:

C_{st_1} mg/dm³	15.2	S_{st1}	452	C_{IST_1} mg/dm³	28.9	S_{IST_1}	3120
C_{st_2} mg/dm³	21.5	S_{st2}	1766	C_{IST_2} mg/dm³	29.2	S_{IST_2}	3234
C_{st_3} mg/dm³	27.1	S_{st3}	3233	C_{IST_3} mg/dm³	27.8	S_{IST_3}	3167
C_{st_4} mg/dm³	33.2	S_{st4}	4127	C_{IST_4} mg/dm³	26.9	S_{IST_4}	3222
C_{st_5} mg/dm³	40.1	S_{st5}	5623	C_{IST_5} mg/dm³	29.6	S_{IST_5}	3098
		S_x	1456	C_{IST_x} mg/dm³	29.5	S_{IST_x}	3145

SOLUTION:

Calculate the ratios of analyte to IST signals (S_{st_n}/S_{IST_n}) and analyte to IST concentration (C_{st_n}/C_{IST}) for each of the standard solutions.

Calculate regression parameters of the function $S_{st\_n}/S_{IST\_n} = f(C_{st\_n}/C_{IST\_n})$.

For the data obtained:

S_{st_n}/S_{IST_n}	C_{st_n}/C_{IST_n}
0.145	0.526
0.546	0.736
1.021	0.975
1.281	1.234
1.815	1.355

Using the linear regression method, the slope and intercept values were calculated.

$$S_{st\_n}/S_{IST} = b \times \left(C_{st\_n}/C_{IST\_n}\right) + a$$

the slope $b = 1.86$

the intercept $a = -0.83$

The concentration of the analyte in the test sample has been calculated according to the following formula:

$$C_x = \left(\frac{\frac{S_x}{S_{IST\_x}} - a}{b} \right) \times C_{IST\_x}$$

Conclusion: The calculated analyte concentration in the test sample was C_x = 20.6 mg/dm^3.

Excel file: exampl_8_12.xls

8.3.6.1 Isotope Dilution Mass Spectrometry Technique The isotope dilution mass spectrometry (IDMS) technique represents a particular variant of the internal standard technique. Its distinctive feature is that the substance introduced is a known quantity of the compound, which differs from the analyte solely in terms of its isotopic composition. During the quantitative analysis, the signal ratios for the corresponding mass ions (of at least two) obtained during the analysis of the actual sample, the standard sample and the actual sample with the addition of the standard are determined.

In order to ascertain the analyte content of the tested sample, it is sufficient to be aware of the quantity of the isotopically determined analyte that has been introduced to the sample. As the quantity of the incorporated standard can be ascertained through the utilization of one of the principal methodologies (gravimetry or volumetry), this serves as the foundation for the incorporation of the IDMS technique within the category of primary methodologies.

The analyte content is determined on the basis of the signal ratios of the corresponding mass ions (typically two ions) present in the tested sample, standard and sample with the standard added. This is done in accordance with the relationship [8], which requires the knowledge of the amount of the added, isotope-determined standard:

$$n_{smpl} = \frac{\left(R_{smpl\&st} - R_{st} \right)}{\left(R_{smpl} - R_{smpl\&st} \right)} \cdot n_{st} \tag{8.4}$$

where:

n_{smpl} – the amount of analyte in the sample,
n_{st} – the amount of isotopically labeled standard added to the sample,
R_{smpl} – ratio of mass ion signals in the test sample,
R_{st} – ratio of mass ion signals in the sample of the standard,
$R_{smpl\&st}$ – ratio of mass ion signals in the sample with added standard.

It is crucial to note that from the moment of adding the standard to the final determination, both the analyte and its isotopically determined counterpart are subjected to the same environmental influences during the storage and preparation of the sample for analysis. This is exemplified by the fact that the extraction of the analyte from the matrix occurs with the same force and that the percentage of losses incurred during the purification of the extract are identical. These factors collectively ensure the stability of the concentration ratio of the analyte and its isotopically determined counterpart throughout the analytical procedure. The accuracy of the IDMS results is independent of the recovery value, as achieving and maintaining a balance between the analyte present in the real sample and the isotope-labelled analogue added in the standard is achieved. It should be noted that this assertion is only valid when the signal value obtained for the determined analyte is higher (despite the low recovery value) than the determined value of the limit of quantification of the methodology.

It is also important to highlight that the feasibility of performing determinations using the IDMS technique is contingent upon the availability of a specific isotope equivalent of the analyte of appropriate purity and the capacity to determine the amount of the analyte added to the sample with an appropriate level of accuracy.

In order to employ the IDMS technique in analytical practice, the following conditions must be met [9]:

- isotope-labeled analogues of the analyte are available (of appropriate purity and, above all, durability);
- it is possible to measure the quantity of the standard with satisfactory accuracy, precision and possibly low and, above all, known uncertainty; all known sources of uncertainty must be accounted for,

- the state of equilibrium between the analyte and its isotopically determined counterpart must be reached (this is the fundamental assumption of the IDMS technique),
- the addition of the standard to the sample does not cause significant changes in the composition of the matrix.

8.4 Conclusions

In conclusion, calibration represents an essential component of any analytical procedure. The objective of calibration is to minimize measurement errors, thereby ensuring the quality and reliability of the results obtained (quality assurance and quality control [QA/QC]). Furthermore, calibration plays a pivotal role in the development of novel analytical procedures and in assessing their scope of applicability. This is because, prior to their introduction into analytical practice, such procedures must undergo validation in the laboratory through the utilization of appropriate reference samples in model tests.

References

1. Webster's Seventh New Collegiate Dictionary, G. & C. Merriam Co., Springfield, MA (1970).
2. Bonate P.L., LC –GC, 10, 310 (1991).
3. Guidelines for Calibration in Analytical Chemistry, Part 1: fundamentals and single component calibration. IUPAC Recommendation. *Pure. Appl. Chem*., 70, 993 (1998).
4. Świtaj-Zawadka A., Konieczka P., Przyk E., and Namieśnik J., *Anal. Lett*., 38, 352 (2005).
5. Drozd J., and Novak J., *J. Chromatogr*., 16, 309 (1982).
6. Kościelniak P., *Chemom. Intell. Lab. System*, 47, 275 (1999).
7. Kościelniak P., *Calibration in Analytical Science. Methods and Procedures*, Wiley-VCH Verlag GmbH, 2023.
8. Kipphardt H., De Bièvre P., and Taylor P.D.P., *Anal. Bioanal. Chem*., 378, 330 (2004).
9. Stargent M., Harrington C., and Harte R., *Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry (IDMS)*, Royal Society of Chemistry, 2002.

9
Method Validation

9.1 Introduction

Considerations concerning the determination of validation parameters should begin with explanation and description of the nature of an analytical measurement. The key interests of analysts worldwide are the signals following and resulting from a conducted measurement. The goal of an analyst's work is to obtain analytical information about an investigated object based on a received output signal, a result of a suitable measurement method. This signal reveals information about the investigated sample. The analyst's role is to "decode" the obtained signal and do it in a manner such that the obtained information is as reliable as possible [1]. A tool that decodes information is an analytical process, including analytical methods applied in the process.

Each signal is characterized by a particular quantity. In some measurements, a signal may also be assigned a position (location). Validation parameters are determined based on the analysis of the obtained signal values, and one should be aware of this in the validation of any analytical method.

Validation of an analytical method includes testing of its important characteristics. The final aim is to be certain that the analysis process is reliable and precise, remains under total control of the operator and leads to reliable results.

First of all, validation allows definition of a given analytical method. Using the determined parameters, in the validation process, there exists the possibility of estimating the usefulness (range of use) for a given method and then choosing the optimal method.

As previously stated, for the measurement results to be traceable and have an uncertainty value provided, they must be obtained using an analytical method that is subjected to a prior validation process.

Most often, a validation study is carried out when [2, 3]:

- analytical method is being developed,
- tests for the extension of the applicability of a known analytical method are being conducted, for example, determinations of a given analyte, but in samples characterized by a different matrix composition,
- quality control of the applied method showed variability of its parameters over time,
- a given analytical method has to be used in another laboratory (different from the one in which it has already been subjected to the validation process) or using different instruments, or determinations are to be performed by another analyst,
- a comparison of a new analytical method with another, known reference method is being performed.

The parameter range, the determination of which should underlie the validation process for a given analytical method, depends on the following factors [4]:

- the character of an analytical study to be carried out using a given analytical method (qualitative or quantitative analysis, analysis of a single sample or a routine analytical investigation),
- requirements for a given analytical method,
- time and costs, which need to be spent in the validation process.

The parameters considered necessary for the validation of different types of analytical procedures are presented in Table 9.1 [2, 5].

Table 9.1 Parameters Whose Determination Is Necessary for Different Types of Analytical Procedures [2, 5]

		IMPURITY TEST		
PARAMETER	QUALITATIVE ANALYSIS	LIMIT IMPURITY TEST	QUANTITATIVE IMPURITY TEST	ASSAY TEST
Precision	–[a]	–	+	+
Correctness	–	–[a]	+	+
Specificity	+	+	+	+
Limit of detection	–[a]	+	–	–
Limit of quantitation	–[a]	–	+	–
Linearity	–[a]	–	+	+
Measuring range	–[a]	–[a]	+	+
Ruggedness	+	+	+	+

[a] It might be determined.

Table 9.2 List of Analytical Procedure Parameters That Should Be Validated According to the Recommendations of ICH [6] and USP [7, 8]

PARAMETER		ICH	USP
Precision	– Repeatability	+	+
	– Intermediate precision	+	
	– Reproducibility	+	
Accuracy		+	+
Limit of detection		+	+
Limit of quantification		+	+
Specificity/selectivity		+	+
Linearity		+	+
Measuring range		+	+
Robustness			+
Ruggedness			+

The more parameters included in the validation process, the more time one should spend on the process. In addition, the more restrictive the assumptions for the limit values (expected) of the respective parameters, the more often one should test, calibrate or "revalidate" a given analytical method. It is not always necessary to conduct a full analytical method validation. Therefore, one should determine which parameters should be included in the process.

Table 9.2 contains the parameters which, according to the recommendations of the International Conference on Harmonization (ICH) [6] and European and United States Pharmacopeia (USP) [7, 8], should be included in the validation process.

Apart from determining validation parameters, before commencing validation, one should determine the basic features of an analytical method, namely [2]:

- type of the determined component (analyte),
- analyte concentration,
- concentration range,
- type of matrix and its composition,
- presence of interferents,
- existence of top-down regulations and requirements for the examined analytical method,
- type of the expected information (quantitative or qualitative analysis),

- required limits of detection and quantitation,
- expected and required precision and accuracy of the entire method,
- required robustness of the method,
- required instruments; whether the determinations using a given method have to be carried out using a strictly defined measuring instrument or instruments of a similar type,
- possibility of using a method already validated in another laboratory(ies).

A validation process may be conducted in any order; however, it seems most logical to proceed in the following manner [2, 4]:

- determine the selectivity in the analysis of standard solution samples (optimization of the separation conditions and determination of analytes present in the standard solution samples),
- determine the linearity, limits of detection and quantitation and the measuring range,
- determine the repeatability (short-term precision), for example, based on deviations of the obtained retention times and/or chromatographic peak areas,
- determine the intermediate precision,
- determine the selectivity based on the results obtained in the analyses of real samples,
- determine the accuracy/trueness based on the analysis of reference material samples containing an analyte at different concentration levels,
- determine the robustness of a method, for example, based on the results obtained in interlaboratory comparisons.

The validation process requires the use of various tools such as [9]:

- blank samples (including so-called reagent blanks),
- standard solutions (calibration solutions, test samples),
- samples with a known quantity of added analyte (spiked with the analyte),
- (certified) reference materials,
- repetitions,
- statistical processing of the results.

In this book, we need to stress that the method can be subjected to the validation process only when a suitable optimization study has been conducted.

The process of analytical method validation should be completed with the final report, which includes all information concerning the analytical method.

Validation parameter definitions and the manner of their determination are described below.

9.2 Characterization of Validation Parameters

9.2.1 Selectivity

Usually, the first determined validation parameter is selectivity. Using basic logic, before one commences determination of the properties of an analyte based on measurement of the obtained analytical signal, one should make sure that a given signal is due only to the occurrence of an analyte in an investigated sample.

A quite frequent problem is the interchangeable use of the terms selectivity and specificity, although they differ in their essential meaning.

According to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature [10], selectivity is defined as "the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture". Specificity is described by the IUPAC as the "highest selectivity" and recommends not using the term specificity.

Selectivity is thus the ability of a method to differentiate the examined analyte from other substances. This characteristic is mostly a function of the described measurement technique but can fluctuate depending on the class or group of compounds to which the analyte belongs, or the sample matrix. A specific method is one which shows the highest selectivity.

Selectivity can be defined as [11] "the ability of an analytical process to receive signals whose size depends almost entirely on the concentration of the examined analyte present in the sample".

One can also propose a practical definition [9]: "selectivity is the potential for an accurate and precise determination of the occurrence and/or concentrations of an analyte or groups of analytes in the

presence of other components in a real sample under given measurement conditions".

Selectivity is therefore one of the main parameters characterizing and describing an analytical method, especially a trace analysis [12].

From a practical point of view, an analytical measurement is selective when it is possible to differentiate measurement signals and assign to them respective properties for a given analyte. This undoubtedly depends on the parameters of the obtained signal. If the signal is characterized only by its intensity, one should prove that its size depends only on the investigated properties of a given object. For example, if the mass of a sample is being determined using an analytical balance, then an analyst must be certain that the measured value is due to the real mass of a sample and not, for example, refuse on the balance's tray. This example shows that problems related to selectivity are also linked with direct measurements.

A different situation is observed concerning selectivity when signals are characterized by an additional parameter – position (place). Such a situation takes place in chromatography for example, where retention time additionally characterizes the output signal and assigns it to a specific analyte. In such a case, it becomes necessary to determine the smallest differences between the positions for each analyte, for which the distinction between the obtained signals is possible.

The requirement of selectivity for a measurement process depends first of all on the composition of an analyzed sample [11]. Selectivity is more difficult to obtain:

- the more unknown the sample composition is,
- the more complex the sample's matrix composition is,
- the more similar the properties of the matrix components,
- the greater the number of analytes,
- the smaller the analyte concentration,
- the greater the resemblance between analytes.

An increase in the selectivity of an analysis may be obtained by:

- the use of selective analytical methods,
- elimination of the influence of interferents by removing or concealing them,
- isolation of the analyte from the matrix.

Depending on the type of analytical technique, the various ways of expressing selectivity are different.

9.2.2 Linearity

When an investigated property is certain to be associated with a given signal, one should determine the dependence between these quantities. A linear dependence most frequently occurs in analytical chemistry. The vast majority of analytical measurements use the calibration step, when the output signals are assigned to corresponding analyte concentrations [13]. To determine the functional dependency associating the output signal with analyte concentration, the linear regression method is commonly used. It is also applied in the determination of some validation parameters, such as:

- linearity,
- trueness (based on the value of biases),
- limits of detection and quantitation.

It is also widely used in the calibration of measuring instruments.

Linearity is defined as an interval in the measurement range of an analytical method in which an output signal correlates linearly with the determined analyte concentration.

The most frequent manner of determining linearity is by using a graph of measuring instrument calibration. To this end, measurements of standard solution samples are conducted on at least six levels of concentrations (most often three parallel measurements for each level). Naturally, the selection of analyte concentrations in standard solution samples should be such that their range should include the expected analyte concentration in an investigated sample (the concentration range usually covers values from 50% to 150% in relation with the expected results of an analysis) [14]. Then, using the linear regression method, one determines the regression parameters.

According to some recommendations [15], it is sufficient to calculate the coefficient of regression. Then, if this value is at levels equal to at least 0.999, we may talk about the linearity of the method within the range of concentrations for which standard solutions were prepared to determine the calibration graph.

Unfortunately, this manner of documenting linearity does not always lead to correct conclusions. It can happen that the high value obtained for the coefficient of regression r (or the coefficient of determination r^2) does not necessarily prove the linearity of a method.

The coefficient of regression may be used to infer the linearity of an analytical method only when standard solutions, based on which the calibration curve is determined, fulfill the following requirements [14–17]:

- they include the expected analyte concentration in the investigated sample(s) within their own range of concentrations,
- they include no more than three orders of magnitude of analyte concentrations within their own range,
- they evenly "cover" the whole range of concentrations.

In addition, it is very important to determine a suitable dependence and the "visual" analysis of the obtained graph.

Because of the ambiguity in the usage of the coefficient r as a measure of linearity, additional methods for proving linearity have been proposed.

In addition, the significance of the calibration graph coefficients needs to be determined. The slope should differ statistically and significantly from *0*, and in the case of an intercept, its value should not differ in a statistically significant way from *0*. To ascertain this, one should calculate the values of the Student's t test (Section 1.8.9).

Another approach is to draw a so-called graph of constant response described by the following dependence [2]:

$$\frac{y}{x} = f(x) \tag{9.1}$$

where:

y – signal of a measuring instrument,

x – analyte concentration in a standard sample corresponding to a given signal.

When the range of concentrations is sufficiently large (including three or more orders of magnitude), the concentrations may be marked on the graph in a logarithmical scale. On such a graph, the sustained response is marked (calculated usually as an arithmetical

mean of individual values *y/x*) in the form of a line parallel to the *X*-axis, along with the admissible deviations from this value (most often ±5%). Values (points) lying outside the determined range correspond to analyte concentrations that lie outside the linear range of the measuring instrument.

Naturally, this process can only be used when an intercept of the determined simple dependence $y = f(x)$ does not differ in a statistically significant manner from zero, which is not always the case.

In some studies, one can find unambiguous and categorical statements that the value of coefficient r cannot serve to determine the degree of dependence between variables and should be replaced by another statistical tool or specific tests for proving linearity [18]. One of the recommended tools is variance analysis. One can also use other methods and statistical tools such as [19–22]:

- test of adequacy,
- Mandel's test,
- quality factor,
- Student's *t* test (Section 1.8.9).

When proving linearity is based on the analysis results of the standard solution series with the simultaneous drawing of a calibration graph, it is logical to prove to what extent the calibration curve reflects the signals for standard solution samples. One can ascertain this through the calculation of relative errors for each concentration, with the reference value being the analyte concentration in the standard sample, and the experimental value being that calculated from the equation of a straight calibration line [23].

Linearity by no means signifies that within the entire range of concentrations, the function describing the dependence of the output signal on the analyte concentration assumes one form (the same calibration curve coefficients). Linearity is a characteristic showing the linear dependence of a signal on the determined quantity and can be described, for a given range, by several equations depending on the level of analyte concentrations [24, 25].

It is also necessary to explain the difference between correlation and regression. Correlation describes the degree of connection between two variables, and regression describes the manner of their dependence [18].

Example 9.1

Problem: Draw the calibration curve based on the results of the analyte concentration determination results in six standard solution samples (three independent measurements per each of the solutions). Calculate the regression parameters of the calibration curve.

Make an appropriate graph.

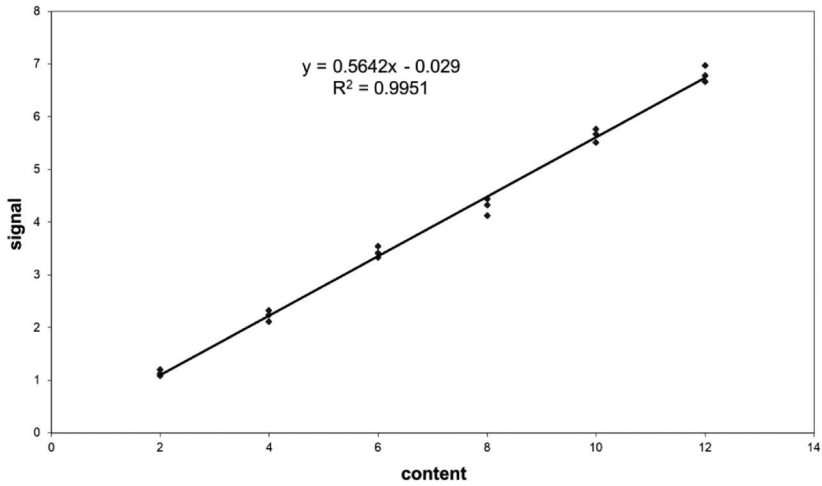
Data: results:

	DATA	
	x	*y*
1	2	1.12
2	2	1.20
3	2	1.08
4	4	2.11
5	4	2.32
6	4	2.23
7	6	3.33
8	6	3.54
9	6	3.41
10	8	4.12
11	8	4.32
12	8	4.44
13	10	5.67
14	10	5.76
15	10	5.51
16	12	6.97
17	12	6.78
18	12	6.66

SOLUTION:

n	18
Slope – *b*	0.5642
Intercept – *a*	–0.029
Residual standard deviation – SD_{xy}	0.14
Standard deviation of the slope – SD_b	0.0099
Standard deviation of the intercept – SD_a	0.077
Regression coefficient – *r*	0.9976

Graph:



Excel file: exampl_9_1.xls

Example 9.2

Problem: Using the data from Example 9.1, examine the significance of the differences in the slope and the intercept of a calibration line and the value 0. Apply the Student's t test.

Calculations should be performed for the significance level $\alpha = 0.05$.

Data: results:

	DATA			DATA	
	x	y		x	y
1	2	1.12	**10**	8	4.12
2	2	1.20	**11**	8	4.32
3	2	1.08	**12**	8	4.44
4	4	2.11	**13**	10	5.67
5	4	2.32	**14**	10	5.76
6	4	2.23	**15**	10	5.51
7	6	3.33	**16**	12	6.97
8	6	3.54	**17**	12	6.78
9	6	3.41	**18**	12	6.66

SOLUTION:

n	18
Slope – b	0.5642
Intercept – a	–0.029
Residual standard deviation – SD_{xy}	0.14
Standard deviation of the slope – SD_b	0.0099
Standard deviation of the intercept – SD_a	0.077
Regression coefficient – r	0.9976
t_b	57.062
t_a	0.378
t_{crit}	2.120

Conclusions:

Statistically significant difference between the slope and 0.

No statistically significant difference between the intercept and 0.

Excel file: exampl_9_2.xls

Example 9.3

Problem: Using the data from Example 9.1, draw a graph of sustained response, marking the lines of the interval for the values deviating ±5% from the mean.

Data: results:

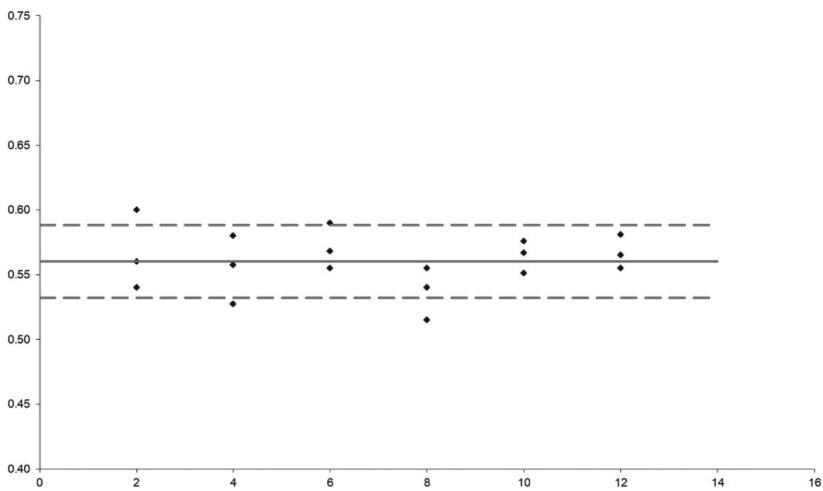
	DATA			DATA	
	x	y		x	y
1	2	1.12	**10**	8	4.12
2	2	1.20	**11**	8	4.32
3	2	1.08	**12**	8	4.44
4	4	2.11	**13**	10	5.67
5	4	2.32	**14**	10	5.76
6	4	2.23	**15**	10	5.51
7	6	3.33	**16**	12	6.97
8	6	3.54	**17**	12	6.78
9	6	3.41	**18**	12	6.66

SOLUTION:

	y/x
1	0.56
2	0.60
3	0.54
4	0.53
5	0.58
6	0.56
7	0.56
8	0.59
9	0.57
10	0.52
11	0.54
12	0.56
13	0.57
14	0.58
15	0.55
16	0.58
17	0.57
18	0.56

x_m	x_m − interval%	x_m + interval%
0.56	0.53	0.59

Graph:



Excel file: exampl_9_3.xls

Example 9.4

Problem: Using the data from Example 9.1, calculate the values of the relative errors for individual values x, assuming the reference value to be x, and the experimental value to be the value calculated from the calibration curve equation.

Assume an appropriate limit for the relative error and draw conclusions.

Data: results:

	DATA	
	x	y
1	2	1.12
2	2	1.20
3	2	1.08
4	4	2.11
5	4	2.32
6	4	2.23
7	6	3.33
8	6	3.54
9	6	3.41
10	8	4.12
11	8	4.32
12	8	4.44
13	10	5.67
14	10	5.76
15	10	5.51
16	12	6.97
17	12	6.78
18	12	6.66

Relative error – ε, %	5.00

SOLUTION:

Number of results – n	18
Slope – b	0.5642
Intercept – a	−0.029

	ε, %	CONCLUSION
1	1.83	OK
2	8.92	!!!
3	−1.72	OK
4	−5.22	!!!
5	4.08	OK
6	0.10	OK
7	−0.78	OK
8	5.43	!!!
9	1.59	OK
10	−8.08	!!!
11	−3.65	OK
12	−0.99	OK
13	1.01	OK
14	2.60	OK
15	−1.83	OK
16	3.37	OK
17	0.56	OK
18	−1.21	OK

Excel file: exampl_9_4.xls

9.2.3 Limit of Detection and Limit of Quantitation

The next validation parameters that need to be determined are the limit of detection (LOD) and the limit of quantitation (LOQ). The values of these parameters are closely related to the magnitude of noises in the measurement system.

Signal to noise ratio (*S/N*) is a unidimensional quantity which describes the relationship of an analytical signal to the mean noise levels for a specific sample. The value of this parameter can serve to determine the influence of noise level on the relative measurement deviation. It can be calculated in different ways, but the most common method is the relationship of the arithmetical mean of the results in a measurement series for blank samples (or samples containing analyte in a very low level) to the standard deviation obtained for this series.

LOD is the lowest concentration (smallest quantity) of an analyte than can be detected with statistically significant certainty [26]; this value is *n*-times the noise level – it is most often three times as high.

Method detection limit (*MDL*) is the lowest concentration (smallest quantity) of an analyte that can be detected using a given analytical procedure.

Instrumental detection limit (e.g. detector) (*IDL*) is the lowest concentration (smallest quantity) of an analyte which can be detected (without quantitative determination) using a given measuring instrument.

LOQ is the quantity or the smallest concentration of a substance that can be determined using a given analytical procedure with an assumed accuracy, precision and uncertainty. This value should be estimated using a suitable standard sample and should not be determined through extrapolation [27].

LOD and LOQ are parameters which play an unusually significant role in the validation of analytical procedures. Although the meaning of these parameters and their understanding do not raise questions, the determination of their values itself is sometimes problematic. This can be attributed to several reasons:

- a large number of definitions describing the notions of both the LOD and the LOQ,
- practical difficulties in univocally determining the basic parameter deciding the LOD – namely, the magnitude of the noise level in a given measuring instrument.

The manner of determining an LOD depends on the following factors:

- nature of the analytical method (the manual method and the method based on utilization of a suitable gauge as well),
- characteristics of the applied instrumental technique,
- possibilities of obtaining (producing) so-called blank samples.

Depending on these parameters, there exist several ways of determining (estimating) the LOD.

9.2.3.1 Visual Estimation For a classical method (noninstrumental) for which it is not possible to determine the noise level of the applied measuring instrument, one estimates the LOD based on one's own experiment. Based on the results of sample analysis with the known analyte concentration (standard solutions), one estimates this

concentration level at which detection is possible. This method can also be used for instrumental techniques.

9.2.3.2 Calculation of LOD Based on the Numerical Value of the S/N Ratio When calculating the LOD, one uses the determined S/N ratio for the investigated analytical procedure [2]. This method can be applied only when it is possible to obtain the baseline of noises, obtained when a blank sample is subjected to final determination.

In this instance, the simplest and most commonly applied way of calculating the LOD is to determine the S/N ratio for a blank sample (if it is possible) or for a sample with a very low analyte concentration, and then to directly apply the principle that LOD is three times the noise level for an applied analytical method.

In the case of chromatography, one can determine the *LOD* value using the obtained chromatogram for a blank sample. To this end, one describes the noise level – measuring range signal changes close to the retention time for an analyte on a chromatogram (one can assume the retention time range as $t_{R\,an} \pm$ of 0.5 min). This quantity is then multiplied by 3, and the obtained signal value is converted into a concentration.

9.2.3.3 Calculation of LOD Based on Determinations for Blank Samples A more labor-consuming method, but one that is also metrologically more correct, uses a measurement for a series of blank samples. It involves 10 independent measurements for 10 independently prepared blank samples [28].

For the thusly obtained 10 results, one calculates the mean value and the standard deviation. LOD is equal to the mean value magnified by three times the standard deviation in this instance.

$$LOD = x_m + 3 \cdot SD \tag{9.2}$$

where:

x_m – mean value,
SD – standard deviation.

In practice, however, it is seldom possible to obtain a numerical value for the mean; it seems paradoxical to obtain a result for a value which by definition should be a submarginal quantitation. The method

would only have some application when the analyte concentration was measurable for a blank sample, that is, the so-called background level is above the LOD for the applied detector (i.e. the analyte concentration in a blank sample is at least equal to the LOQ for the applied detector).

Otherwise, it is possible to use the described method with a certain modification [28, 29] – namely, 10 independent determinations are performed for samples in which the analyte concentration is close to the expected LOD; of course, such samples are prepared through spike in the blank samples with quantifiable amounts of the analyte. The manner of conduct is then similar to the previously described one, with the one difference being that the LOD is calculated according to the formula:

$$LOD = 0 + 3 \cdot SD \tag{9.3}$$

The modification is the preparation of n samples with analyte concentrations on a level close to the expected LOD. Of course, it would be most convenient to prepare standard solutions in which matrix compositions correspond to the matrix composition of real samples. One then performs an analysis on such prepared samples, receiving a series of n results for which one calculates the mean value and standard deviation. LOD is calculated using a dependence described by an equation for the number of degrees of freedom $f = n - 1$, where n is the number of independent samples and the accepted level of significance α.

$$LOD = t \cdot SD \tag{9.4}$$

where:

t – parameter of the Student's t test,

SD – standard deviation.

If the prepared standard solution samples are subjected to analysis using a given analytical procedure, then the determined LOD is also the *MDL*. If determinations are instead performed directly on the prepared standard solution samples, then *IDL* are determined in this manner.

9.2.3.4 Graphical Method This method involves analyses of measurement series for three standard solution samples containing an analyte

at three levels of concentration (close to the expected LOD for the samples). For each level of analyte concentration, one should perform at least six parallel determinations, and then for each series of measurements obtained in this way, calculate the standard deviations. A linear dependence is determined which associates the calculated standard deviations with the respective concentrations:

$$SD = f(c) \tag{9.5}$$

Then, one determines the absolute term SD_o after which one determines the LOD according to the following dependence:

$$LOD = 3 \cdot SD_o \tag{9.6}$$

9.2.3.5 Calculating LOD Based on the Standard Deviation of Signals and the Slope of the Calibration Curve One most often applies analytical methods in which the final determination is based on the indirect measurement principle. In this case, it is indispensable to perform calibration that will influence the LOD [2, 6].

In this case, LOD is calculated using the following dependence:

$$LOD = \frac{3.3 \cdot SD}{b} \tag{9.7}$$

where:

b is the slope of calibration curve.

Standard deviation can be determined in three different ways:

- as a standard deviation of results obtained for the series of blank samples – SD_{bl},
- as a residual standard deviation of the calibration curve – SD_{xy}, described by the dependence (1.68),
- as a standard deviation of the intercept of the obtained calibration curve – SD_a.

Of course, the limit of detection (for the analytical method or the applied detector) will be calculated depending on which parameters were used to calculate the standard deviation. Hence, if measurements are conducted based on analyses of blank samples subjected to the whole analytical procedure, the *MDL* is the determined quantity.

When the LOD is calculated based on parameters of the determined calibration graph (residual standard deviation or standard deviation of the intercept), the calculated value is the LOD for the measuring instrument. It is also important to appropriately select concentrations of standard solutions to draw the calibration graph (it is known that the calibration graph has a straight-line range in a strictly specific interval of concentrations and that its plot most likely has different concentration level characteristics close to the LOD).

9.2.3.6 Calculation of LOD Based on a Given LOQ LOQ is the lowest analyte concentration which can be determined with a suitable precision and accuracy. One performs measurements for standard solutions (matrix standards) on at least five levels of concentrations [2, 28]. For each solution, one performs six parallel measurements. For each level of concentrations, the coefficient of variation (CV) is calculated, and the graph of the *f(c)* dependence is drawn. The required precision for the LOQ is determined (usually = 10%), and for this value, the concentration equal to the LOQ is read on the graph. The LOD is calculated as: $LOD = LOQ/3$.

Figure 9.1 presents the construction of the graph and the calculation of the LOQ [28].

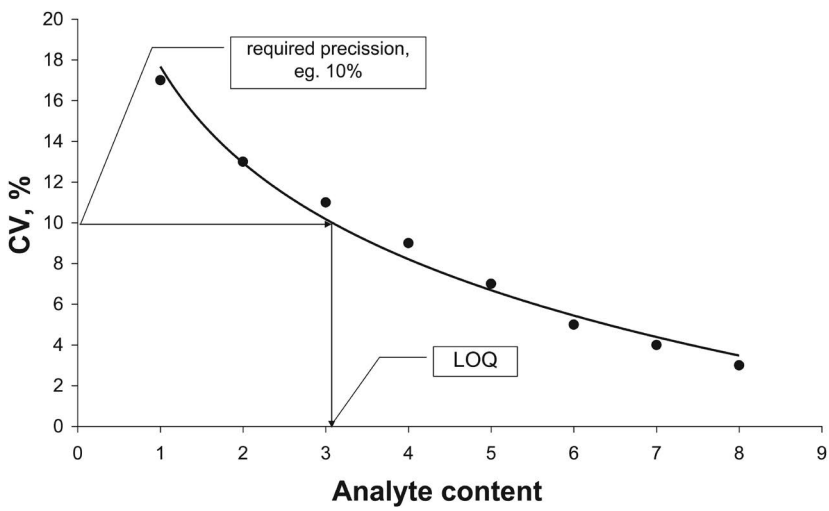


Figure 9.1 Construction of the graph and calculation of the limit of quantitation [28].

Table 9.3 Methods for Determining Detection Limits: Requirements, Disadvantages and Advantages [27]

METHODS FOR CALCULATING *LOD*	REQUIREMENTS	DISADVANTAGES/ADVANTAGES
Visual check	Sample with known analyte content (standard solution or matrix standard)	Quick method Estimation Mostly used in case of classical analysis (non-instrumental) Requires vast analytical experience
Calculations based on the S/N ratio	Sample with known analyte content (standard solution or matrix standard)	Quick method Used only for measuring equipment It is possible to determine the S/N ratio
Calculations based on the measurements for sample blanks	Series of blanks or samples with known analyte content (standard solution or matrix standard)	Labor- and time-consuming method that does not consider the influence of calibration on LOD Probability is used for estimating LOD
Calculations based on graphical method	Series of standard samples at three concentration levels, at least six measurements for each standard sample	Relatively quick method It includes the influence of calibration procedure on LOD value
Calculations based on standard deviations of signals and slope of calibration curve	Series of blanks or samples with known analyte content (standard solution or matrix standard) Standard solutions for calibration curve preparation	Labor- and time-consuming method It includes the influence of calibration procedure on LOD value Method "motivated" by metrology
Calculations based on limit of quantification, LOQ	Series of standard solutions Assumed relative standard deviation for LOQ	Indirect method LOD calculated based on the determined LOQ LOD value (LOQ) depends on the assumed measurement precision

Table 9.3 compares all the described methods of calculating the LOD, together with their short characterizations [27].

9.2.3.7 Testing the Correctness of the Determined LOD Many of the aforementioned ways of calculating the LOD are based on the determination of analyte concentration in the prepared standard solution samples. The solutions should be characterized, while calculating the LOD of an analytical procedure, with two basic features:

- matrix composition should be as close to the matrix composition of real samples as possible,
- analyte concentration should be on a level close to the expected LOD.

It is known that the standard deviation for the set of measurement results determining the analyte concentrations in standard solution samples strictly depends on the concentration levels of a determined component. It can happen that the concentrations in standard samples are considerably higher than the calculated LOD. To check the calculated LOD, one should fulfill the following conditions [29]:

$$10 \cdot LOD > c_{min} \tag{9.8}$$

$$LOD < c_{min} \tag{9.9}$$

where:

c_{min} – the analyte concentration in a standard solution sample with the lowest concentration.

If condition (9.8) is not fulfilled, it will signify that the concentration in the prepared standard samples is too high. One should then calculate the LOD for newly prepared standard solutions with a lower analyte concentration. Inversely, when the condition (9.9.) is not fulfilled, the analyte concentration in the prepared standard samples is too low. In this case, one should remeasure and recalculate using standard solutions in which the analyte occurs in higher concentration levels.

In order to test the trueness of the calculated LOD, one can also estimate the S/N ratio based on the following dependence [29].

$$S/N = \frac{x_m}{SD} \tag{9.10}$$

According to the definition of LOD, the numerical value of this ratio should be between 3 and 10. When it is higher, the determined *LOD* is greater than the numerical value, and one should conduct remeasurement for lower concentrations of the analyte in standard solution samples.

One should also pay attention to the recovery of the analytical method in measurements conducted for standard solutions. Recovery can be calculated using the dependence [29]:

$$\%R = \frac{x_m}{c}[\%] \tag{9.11}$$

where:

%R – recovery of an analyte for a given analytical procedure.

A recovery being too low results in an undervaluation of the calculated LOD.

As previously stated, the described methods of testing the correctness of the calculated LOD can only be applied when the measurements are performed using prepared standard solutions.

The described ways of determining the LOD and/or quantitation permit the determination of both the MDL and the IDL.

The choice of a suitable means for determining the LOD depends on the purpose of the limit and the requirements of a given analytical method. For the validation of an analytical method, it is recommended to use a way the assumptions of which are based on chemical metrology; the value of the determined LOD is associated with statistical parameters such as:

- level of probability,
- number of degrees of freedom.

For individual measurements, it is recommended to apply a less time-consuming method.

It must be stated that the determined LOD should always be given the description and parameters of the method applied in its calculation.

The determined limits of detection and quantitation also show the quality of measurements conducted using a given analytical method [30–32].

Determining limits of detection and quantitation allows the unequivocal determination and presentation of results in the proximity of these values. A correct method for recording a determination result depending on the quantity of an analytical signal is presented in Table 9.4.

It has to be stressed that both LOD and LOQ are the parameters which are estimated. It means that its presentation should have a maximum of two significant digits.

Table 9.4 Correct Method for Recording a Determination Result

RESULT, x	RECORDING OF RESULT
$x < LOD$	Not determined
$LOD \leq x < LOQ$	Not quantified
$x \geq LOQ$	Value of concentration

Example 9.5

Problem: Using the given analyte concentration determinations for blank samples, estimate *LOD* and *LOQ* for the validated analytical method.

Using the calculated S/N ratio, examine the correctness of the determined *LOD*.

Data: results, ng/g:

	DATA
1	0.155
2	0.132
3	0.143
4	0.121
5	0.145
6	0.113
7	0.137

SOLUTION:

x_m	0.135	ng/g
SD	0.014	ng/g
LOD	0.18	ng/g
LOQ	0.54	ng/g

$$LOD = x_m + 3 \cdot SD$$

$$S/N = \frac{x_m}{SD}$$

S/N	9

Conclusion: The *S/N* ratio is in the range 3 ÷ 10, therefore the calculated *LOD* is correct.

Excel file: exampl_9_5.xls

Example 9.6

Problem: When the measurements were performed on blank samples, it was noticed that the obtained values of signals cannot be measured. Hence, the standard solutions were made with concentrations near the expected *LOD*, and based on the measurements for these solutions, the estimation was made for *LOD* and *LOQ*.

Check the correctness of the *LOD* determination through the comparison with the standard solution concentration.

Data: results, ng/g:

	DATA
1	0.235
2	0.253
3	0.258
4	0.254
5	0.244
6	0.258
c	**0.250**

SOLUTION:

SD	0.0091	ng/g
LOD	0.027	ng/g
LOQ	0.082	ng/g

$$LOD = 0 + 3 \cdot SD$$

$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

Conclusion: Calculated *LOD* is lower than standard solution concentration used for its determination, and 10 times *LOD* is higher than standard solution concentration, calculated *LOD* is correct.

Excel file: exampl_9_6.xls

Example 9.7

Problem: Using the data given determinations of the analyte concentrations for blank samples, estimate the *LOD* and *LOQ* of the validated analytical method, using the Student's *t* test.

Using the data calculated S/N ratio, check the correctness of the determined LOD.

Data: results, mg/dm^3:

	DATA
1	8.8
2	7.6
3	9.2
4	9.5
5	6.8
6	7.4
7	9.6
α	0.05

SOLUTION:

x_m	8.41	mg/dm^3
SD	1.13	mg/dm^3
t	2.447	
LOD	2.8	mg/dm^3
LOQ	8.3	mg/dm^3

$$LOD = t \cdot SD$$

$$S/N = \frac{x_m}{SD}$$

S/N	7

Conclusion: *S/N* ratio is in the range 3 ÷ 10, and the calculated *LOD* is correct.

Excel file: exampl_9_7.xls

Example 9.8

Problem: Using the given analyte concentration determinations for standard solution samples, estimate the *LOD* and *LOQ* using a graphical method. Draw an appropriate graph. Present *LOD* in units of the analyte concentration in standard solutions applied for *LOD* estimation.

In addition, check the correctness of the *LOD* determination through a comparison with the standard solution with the lowest concentration.

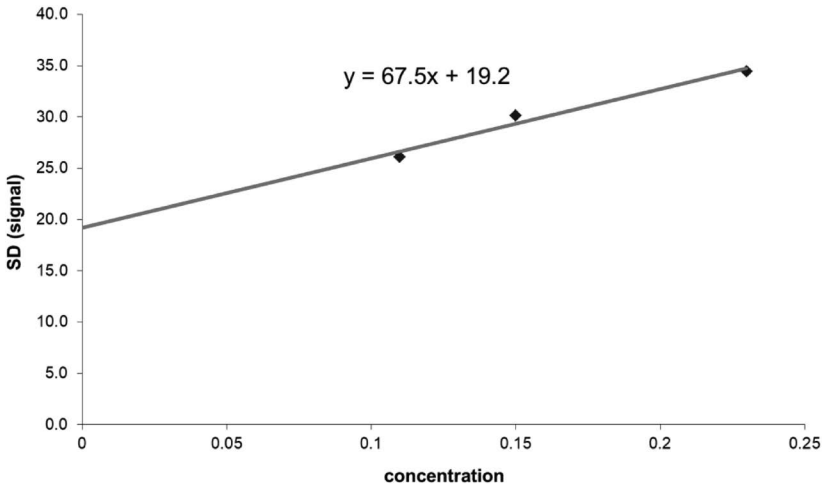
Data: results:

	CONCENTRATION, ppm		
	0.11	0.15	0.23
		SIGNALS	
1	101	198	298
2	144	177	237
3	124	132	222
4	174	156	257
5	102	205	243
6	111	193	313
7	121	135	235

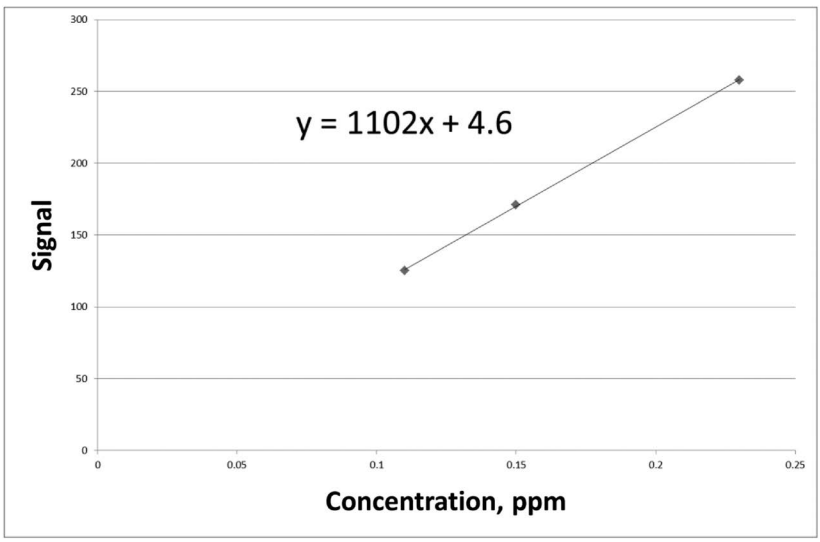
SOLUTION:

CONCENTRATION	SIGNAL	*SD* (SIGNAL)
0.11	125.3	26.1
0.15	170.9	30.1
0.23	257.9	34.4
SD_o	19.2	Signal
LOD	0.013	ppm
LOQ	0.040	ppm

Graph:



Graph_calibration:



$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

Conclusion: Calculated *LOD* is lower than the lower concentrated standard solution used for its determination, and 10 times *LOD* is higher than the lower concentrated standard solution; the calculated *LOD* is correct.

Excel file: exampl_9_8.xls

Example 9.9

Problem: Using the data from Example 9.8, estimate the *LOD* and *LOQ* via the method using parameters of the calibration curve.

Present the value of *LOD* in the units of standard solution concentration, applied in *LOD* estimation.

Also check the correctness of *LOD* determination, comparing the calculated value with the value of the analyte concentration in the standard solution with the lowest concentration.

Data: results:

	CONCENTRATION, ppm	SIGNAL
1	0.11	101
2	0.11	144
3	0.11	124
4	0.11	174
5	0.11	102
6	0.11	111
7	0.11	121
8	0.15	198
9	0.15	177
10	0.15	132
11	0.15	156
12	0.15	205
13	0.15	193
14	0.15	135
15	0.23	298
16	0.23	237
17	0.23	222
18	0.23	257
19	0.23	243
20	0.23	313
21	0.23	235

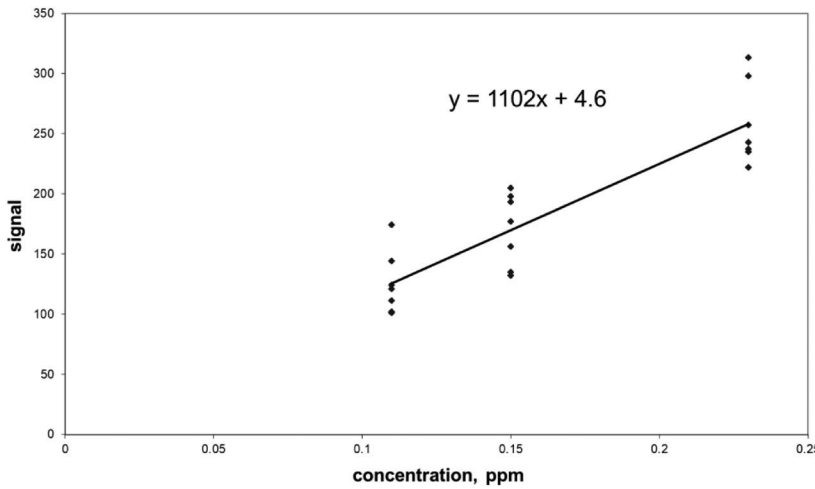
SOLUTION:

Number of results – *n*	21
Slope – *b*	1102
Intercept – *a*	4.6
Residual standard deviation – SD_{xy}	29.6
Standard deviation – SD_b	129
Standard deviation – SD_a	22.1
Regression coefficient – *r*	0.8901

***LOD* ($SD_{x,y}$)**	0.089	ppm
***LOD* (SD_a)**	0.066	ppm
***LOD* (mean)**	0.077	ppm

$$LOD = \frac{3.3 \cdot SD}{b}$$

Graph:



$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

Conclusion: Calculated *LOD* is lower than the lower concentrated standard solution used for its determination, and 10 times *LOD* is higher than the lower concentrated standard solution; calculated *LOD* is correct.

Excel file: exampl_9_9.xls

Example 9.10

Problem: Using the analyte concentration determinations for standard solution samples, estimate the *LOD* and *LOQ* by a method using the parameters of the calibration curve.

Present the values of *LOD* in units of standard solution concentrations applied for *LOD* determination.

Also check the correctness of *LOD* determination comparing the calculated value with the analyte concentration in a standard solution with the lowest concentration.

Data: results:

	CONCENTRATION, ppm	SIGNAL
1	1.2	1460
2	1.2	1725
3	1.2	1150
4	1.2	1025
5	1.2	1825
6	1.2	1310
7	2.5	1950
8	2.5	1630
9	2.5	2200
10	2.5	1650
11	2.5	2000
12	2.5	1980
13	3.3	2900
14	3.3	3200
15	3.3	3245
16	3.3	2850
17	3.3	3500
18	3.3	3890

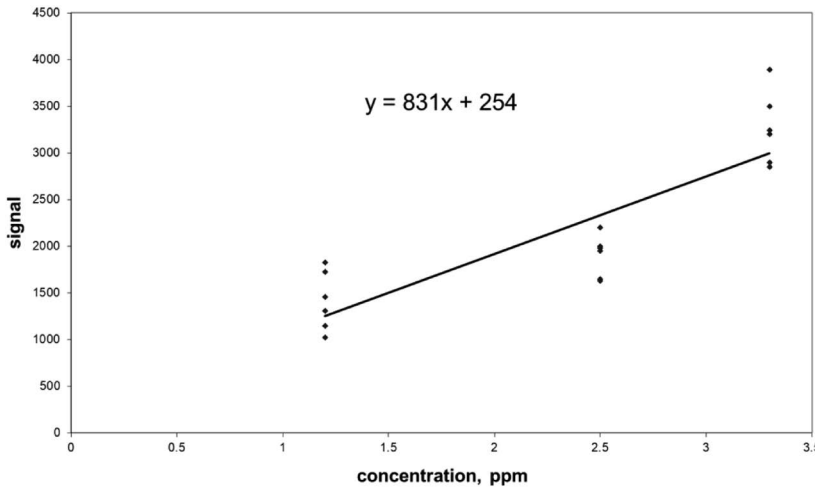
SOLUTION:

Number of results – *n*	18
Slope – *b*	831
Intercept – *a*	254
Residual standard deviation – SD_{xy}	447
Standard deviation – SD_b	122
Standard deviation – SD_a	303
Regression coefficient – *r*	0.8627

LOD (SD_{xy})	1.8	ppm
LOD (SD_a)	1.2	ppm
LOD (mean)	1.5	ppm

$$LOD = \frac{3.3 \cdot SD}{b}$$

Graph:



$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

Conclusion: Because the concentration of a solution with the lowest concentration is lower than the calculated LOD, standard solutions with a higher concentration were made, and new calculations were made for the new series of data (without measurements for the solution with the lowest concentration).

Data (2): results:

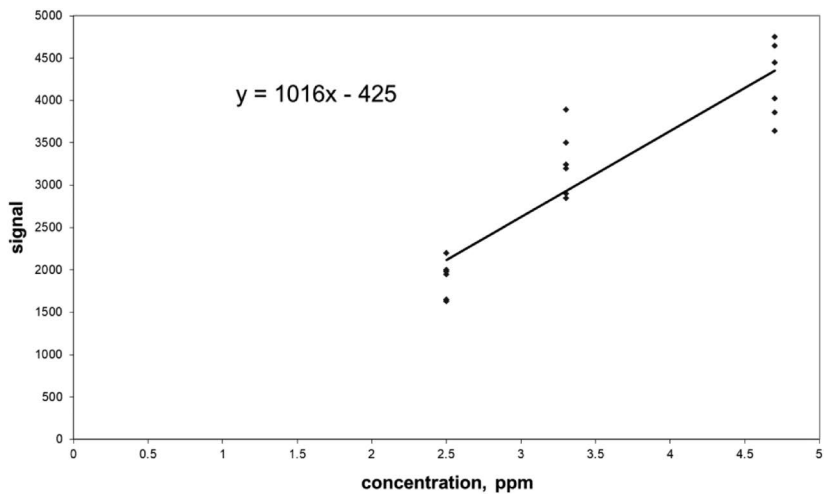
	CONCENTRATION, ppm	SIGNAL		CONCENTRATION, ppm	SIGNAL
1	2.5	1950	**10**	3.3	2850
2	2.5	1630	**11**	3.3	3500
3	2.5	2200	**12**	3.3	3890
4	2.5	1650	**13**	4.7	3640
5	2.5	2000	**14**	4.7	4650
6	2.5	1980	**15**	4.7	3860
7	3.3	2900	**16**	4.7	4750
8	3.3	3200	**17**	4.7	4450
9	3.3	3245	**18**	4.7	4025

SOLUTION (2):

Number of results – *n*	18
Slope – *b*	1016
Intercept – *a*	–425
Residual standard deviation – SD_{xy}	437
Standard deviation – SD_b	113
Standard deviation – SD_a	410
Regression coefficient – *r*	0.9132

***LOD* (SD_{xy})**	1.4	ppm
***LOD* (SD_a)**	1.3	ppm
***LOD* (mean)**	1.4	ppm

Graph (2):



Conclusion (2): Calculated *LOD* is lower than the lower concentrated standard solution used for its determination, and 10 times *LOD* is higher than the lower concentrated standard solution; the calculated *LOD* is correct.

Excel file: exampl_9_10.xls

Example 9.11

Problem: Using the values of the analyte concentration determinations for standard solution samples, estimate the *LOQ* and then the *LOD* using an *LOQ* determination method based on the assumed value of determination precision. Assume the maximum value of the coefficient of variation to be $CV = 5\%$.

Draw an appropriate graph.

Present *LOD* in units of the standard solution concentration applied for *LOD* determinations.

Data: results:

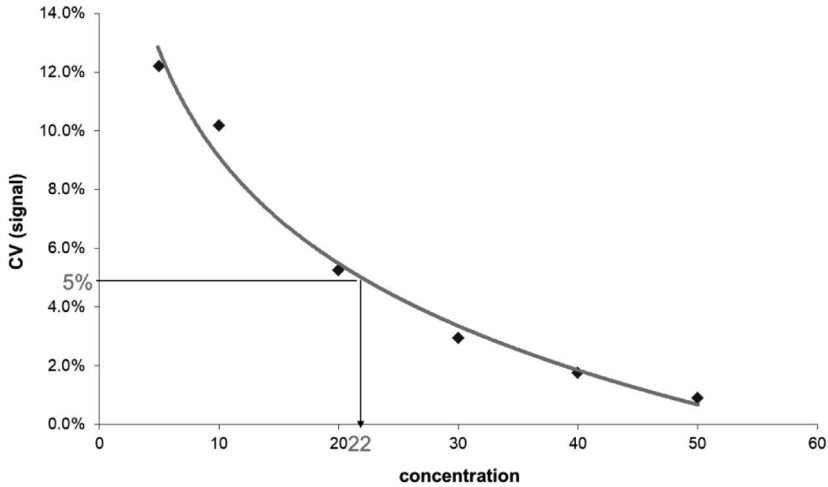
	CONCENTRATION, ppm					
	5.0	**10.0**	**20.0**	**30.0**	**40.0**	**50.0**
	SIGNALS					
1	104	198	444	635	800	1000
2	144	177	450	650	810	990
3	124	232	470	660	805	995
4	124	200	400	620	825	1010
5	102	205	445	610	820	1005
6	111	193	450	625	840	1015
7	121	235	470	615	830	995

SOLUTION:

CONCENTRATION, ppm	*CV*, %
5.0	12.2
10.0	10.2
20.0	5.24
30.0	2.95
40.0	1.75
50.0	0.898

LOQ	22 ppm
LOD	7.3 ppm

Graph:



Excel file: exampl_9_11.xls

9.2.4 Range

Determination of linearity and the LOQ enables the determination of a measuring range for an analytical method. A **measuring range** is a range of values (analyte concentrations) in which the error of a measuring instrument is below the assumed value. In practice, it is described as an interval between the LOQ and the highest analyte concentration for which a measuring system shows an increase in the output signal.

Example 9.12

Problem: Determine the calibration curve based on analyte concentration determinations in eight standard solutions samples (five independent measurements for each solution). Calculate regression parameters of the calibration curve.

Prepare an appropriate graph.

Using the determinations for standard solution samples for three lowest concentration levels, estimate the *LOD* and *LOQ* using a technique based on using parameters of the calibration curve.

Present the *LOD* in units of standard solution concentration applied in *LOD* estimation.

Also check the correctness of *LOD* determination comparing the calculated value with the analyte concentration in the standard solution with the lowest concentration.

Present the measuring range of the analytical method.

Data: results:

	CONCENTRATION, ppb	SIGNAL
1	0.65	780
2	0.65	745
3	0.65	756
4	0.65	770
5	0.65	735
6	1.12	1420
7	1.12	1450
8	1.12	1425
9	1.12	1350
10	1.12	1411
11	2.44	3100
12	2.44	3005
13	2.44	3000
14	2.44	3100
15	2.44	3105
16	3.75	4700
17	3.75	4650
18	3.75	4850
19	3.75	4760
20	3.75	4690
21	5.25	6750
22	5.25	6800
23	5.25	7100
24	5.25	6690
25	5.25	6990
26	7.8	10,100
27	7.8	10,000
28	7.8	9900
29	7.8	10,350
30	7.8	10,150
31	10.4	13,400
32	10.4	13,200
33	10.4	13,300
34	10.4	13,000
35	10.4	12,950
36	13.3	16,600
37	13.3	16,745
38	13.3	16,600
39	13.3	16,200
40	13.3	16,500

SOLUTION (CALIBRATION):

Number of results – *n*	40
Slope – *b*	1255.9
Intercept – *a*	59
Residual standard deviation – SD_{xy}	200
Standard deviation – SD_b	7.4
Standard deviation – SD_a	52
Regression coefficient – *r*	0.9993

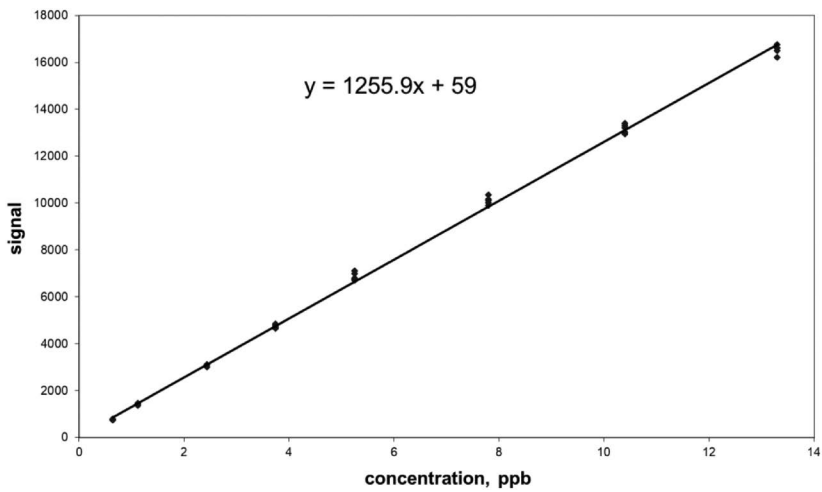
SOLUTION (LOD):

Number of results – *n*	15
Slope – *b*	1280
Intercept – *a*	–52
Residual standard deviation – SD_{xy}	45
Standard deviation – SD_b	15
Standard deviation – SD_a	24
Regression coefficient – *r*	0.9991

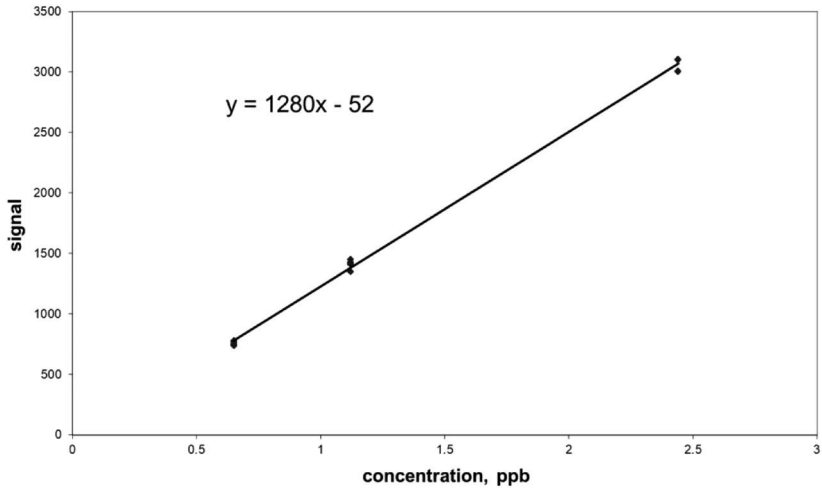
***LOD* (SD_{xy})**	0.12	ppb
***LOD* (SD_a)**	0.063	ppb
LOD (mean)	0.089	ppb
LOQ	0.27	ppb
Range	0.27 ÷ 13.3	ppb

$$LOD = \frac{3.3 \cdot SD}{b}$$

Graph (calibration):



Graph (LOD):



$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

Conclusion: The calculated *LOD* is lower than the lower concentrated standard solution used for its determination, and 10 times *LOD* is higher than the lower concentrated standard solution; the calculated *LOD* is correct.

Excel file: exampl_9_12.xls

9.2.5 Sensitivity

Sensitivity is a parameter that is not a necessary parameter in the validation of an analytical method. One can determine its value based simply on the parameters of the calibration curve. **Sensitivity** is the relationship of change in the output signal of a measuring instrument to the change in the analyte concentration that induces it. Thus, sensitivity shows the smallest difference in the analyte concentration that can be ascertained using a specific method (it is a slope of a calibration graph: signal in the concentration function).

As a recapitulation, Figure 9.2. presents the interpretation of linearity, measuring range, LOD, LOQ and sensitivity [28].

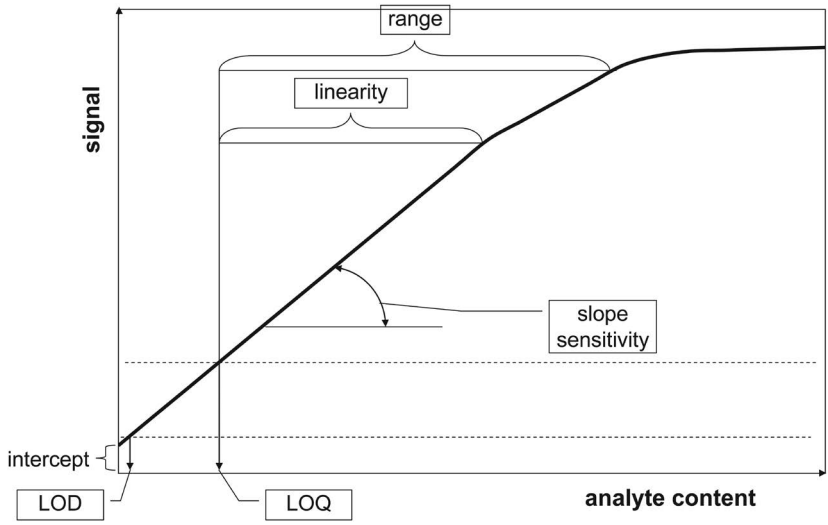


Figure 9.2 Interpretation of linearity, measuring range, limit of detection and limit of quantitation and sensitivity [28].

9.2.6 Precision

Each of the parameters below is determined based on the calculated standard deviation for the series of measurements, and therefore the manner of conduct in their determination will be described together.

Repeatability, intermediate precision and reproducibility can be determined based on the determined standard deviation, relative standard deviation or the so-called coefficient of variation.

Precision is the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions [26].

It is associated with random errors and is a measure of dispersion or scattering around the mean value, usually expressed by a standard deviation.

Repeatability is the measurement precision under a set of repeatability conditions of measurement [26]. The precision of results is obtained under the same measurement conditions (a given laboratory, analyst, measuring instrument, reagents, etc.). It is usually expressed by a repeatability standard deviation, variance, relative standard deviation or coefficient of variation.

Intermediate precision is the precision of results obtained in a given laboratory over a long-term process of measuring. Intermediate precision is a more general notion (due to the possibility of changes in the greater number of determination parameters) compared to repeatability.

Reproducibility is the precision of results obtained by different analysts in different laboratories using a given measurement method.

In determining repeatability, it is recommended for an analysis to be conducted on samples characterized with different analyte concentrations and differing in matrix composition.

According to recommendations by the ICH [6], standard deviation can be calculated in one of the following ways:

- at least nine independent determinations in the whole measuring range (e.g. three independent determinations for three concentration levels),
- six independent determinations of an analyte in standard samples for the concentration level corresponding to the concentration of a real sample,
- six independent determinations of analytes occurring in three different matrices and for two or three concentration levels.

According to EURACHEM recommendations [28], one should perform 10 independent determinations and calculate the standard deviation based on these.

The determined method's repeatability can refer both to (1) a very specific analytical method in which matrix composition is specific and defined (e.g. the method of determining analyte X concentration in matrix Y) and (2) determination methods for a given analyte without specifying matrix composition. In the former case, the standard deviation is calculated based on measurements performed for samples characterized by the same matrix composition. In the latter case, one needs to calculate the standard deviation using the measurements conducted for samples differing in matrix composition.

Intermediate precision is a notion with a wider scope than repeatability because its value is influenced by additional parameters such as [2, 3]:

- personal factors – different analysts conducting determinations and instability in the work of a given analyst over a specific period,

- instrumental factors – due to the fact that measurements can be carried out using:
  - different measuring instruments from a given laboratory,
  - standard solutions and reagents coming from different producers, or from different batches,
- different accessories, for example, different gas chromatography (GC) columns, with the same characteristics but from different producers, or from different batches.

If determining precision uses samples in which analyte concentration is stable, the standard deviation is a sufficient parameter which one may determine precision with. However, in the analysis of samples characterized by different levels of analyte concentration, one should use the relative standard deviation or coefficient of variation. Each of these of two quantities is used to compare repeatability, intermediate precision or reproducibility.

9.2.6.1 Manners of Estimating the Standard Deviation Determining intermediate precision, repeatability and reproducibility is based on calculating the standard deviation for the series of obtained measurement results [33–38]. The simplest means of estimating this parameter is by calculating the relative standard deviation or coefficient of variation and comparing (assessment) the obtained values. Frequently, one can find the statement that if a relative standard deviation (*RSD*) is smaller than a certain determined limit, then using a given method can yield precise results.

An estimation of standard deviation can be performed using suitable statistical tests:

- with a set point of this parameter –χ^2 test (Section 1.8.4),
- with the value obtained from a statistical assessment of the set of results obtained using a reference method – Snedecor's *F* test (Section 1.8.5).

Sometimes, it is necessary to compare the standard deviation for sets of measurement results obtained using more than two methods. If the number of measurements on which the calculation of standard deviations is based is similar for all methods (equinumerous series of measuring), then one can apply the Hartley's F_{max} test (Section 1.8.6).

When the number of results obtained using the compared methods is different, one should compare the calculated standard deviations using *Bartlett's* test (Section 1.8.7).

If the standard deviations are to be compared for two sets of correlated results, one should use Morgan's test (Section 1.8.8).

Example 9.13

Problem: For the given measurement result series, check (at the level of significance $\alpha = 0.05$) if the calculated standard deviation differs statistically significantly from the set value of the standard deviation.

Apply the χ^2 test.

Data: results:

11.0	12.0	12.9	12.0	12.5	12.1	14.2	12.1	17.1	12.1	12.4	15.1	12.3	12.0	10.2

$$SD_o = 1.23$$

SOLUTION:

Number of results – *n*	15
Standard deviation – *SD*	1.68
χ^2	27.88
$\chi^2_{crit}\,(f = 14, \alpha = 0.05)$	23.68

The calculation was performed using Equation 1.29 – Chapter 1, Subsection 1.8.4.

Conclusion: Because $\chi^2 > \chi^2_{crit}$, there is a statistically significant difference in variance value.

Excel file: exampl_9_13.xls

Example 9.14

Problem: For the given series of measurement results, check (at the level of significance $\alpha = 0.05$) if the standard deviation values for both the series are statistically significantly different.

Apply the Snedecor's F test.

Data: result series:

	SERIES 1	SERIES 2
1	10	11
2	12	11
3	13	13
4	14	11
5	18	13
6	15	12
7	17	

SOLUTION:

	SERIES 1		SERIES 2
Number of results – *n*	7		6
Standard deviation – *SD*	2.795		0.983
F		7.85	
F_{crit} ($f_1 = 6$, $f_2 = 5$, $\alpha = 0.05$)		4.95	

The calculation was performed using Equation 1.30 – Chapter 1, Subsection 1.8.5.

SOLUTION_2:

F-Test Two-Sample for Variances		
	Variable 1	*Variable 2*
Mean	14,143	11,833
Variance	7,810	0,967
Observations	7	6
df	6	5
F	8,079	
P(F<=f) one-tail	0,0183	
F Critical one-tail	4,950	

Conclusion: Because $F > F_{crit}$, there is statistically significant difference in variance values for the compared series, the series differ in precision

Excel file: exampl_9_14.xls

Example 9.15

Problem: For the given series of measurement results, check (at the level of significance $\alpha = 0.05$) if the values of the standard deviation for the given series of results are statistically significantly different.

Equinumerous series – apply Hartley's F_{max} test.

Data: result series:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5
1	11	13	10	10	17
2	12	12	13	12	11
3	13	12	14	16	13
4	12	15	12	18	14
5	13	11	13	13	13
6	12	10	14	14	12
7	14	13	11	14	13
8	12	11	12	12	11
9	15	12	17	17	13
10	12	14	14	14	14
11	12	15	17	10	15
12	15	12	12	12	11
13	12	14	11	11	11
14	12	12	12	13	12
15	10	11	14	15	12

SOLUTION:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5
Number of results – *n*	15	15	15	15	15
Standard deviation – *SD*	1.36	1.51	2.02	2.38	1.70
F_{max}			3.09		
F_{maxo} $(k = 5,\ f = 14,\ \alpha = 0.05)$			4.76		

The calculation was performed using Equation 1.31 – Chapter 1, Subsection 1.8.6.

Conclusion: Because $F_{max} < F_{maxo}$, there is no statistically significant difference in variance values for the compared series.

Excel file: exampl_9_15.xls

Example 9.16

Problem: For the given series of measurement results, check (at the level of significance $\alpha = 0.05$) if the values of the standard deviation for a given series of results are statistically significantly different.

Not equinumerous – apply the *Bartlett* test.

Data: result series:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
1	11	13	10	10	17	10
2	12	12	13	12	11	13
3	13	12	14	16	13	14
4	12	15	12	18	14	12
5	13	11	13	13	13	13
6	12	10	14	14	12	14
7	14	13	11	14	13	11
8	12	11	12	12	11	12
9	15	12	17	17	13	17
10	12	14	14	14		14
11	12	15	17	10		17
12	15		12	12		12
13	12		11	11		
14	12		12			
15	10					

SOLUTION:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
Number of results – n	15	11	14	13	9	12
Standard deviation – SD	1.36	1.63	2.08	2.53	1.80	2.14
$1/(n-1)$	0.071	0.100	0.077	0.083	0.125	0.091
$(n-1)\cdot\log(SD^2)$	3.701	4.270	8.245	9.672	4.095	7.257
$(n-1)\cdot SD^2$	25.733	26.727	56.000	76.769	26.000	50.250
c			1.04			
SD^2_0			3.845			
Q			51.22			
$\chi^2_{crit}\,(f=k-1=5,\ \alpha=0.05)$			11.07			

The calculation was performed using Equations 1.32–1.34 – Chapter 1, Subsection 1.8.7.

Conclusion: Because $Q > \chi^2_{crit}$, there is a statistically significant difference in variance values for the compared series.

Excel file: exampl_9_16.xls

Example 9.17

Problem: For the given series of measurement results – dependent variables, check (at the level of significance $\alpha = 0.05$) if the values of

the standard deviation for the given series of results are statistically significantly different.

Apply the Morgan's test.

Data: result series:

	SERIES 1	SERIES 2
1	8.8	9.1
2	9.7	9.8
3	8.9	9.2
4	9.3	9.6
5	8.1	8.2
6	8.9	9.1
7	9.4	9.6
8	9.1	10.1
9	9.2	10.3
10	9.1	9.9
11	8.9	9.7
12	8.2	8.7
13	9.1	9.6

SOLUTION:

r	0.809
SD_1	0.44
SD_2	0.58
L	0.816
t	1.576
t_{crit}	2.201

The calculation was performed using Equations 1.35–1.37 – Chapter 1, Subsection 1.8.8.

Conclusion: Because $t < t_{crit}$, there is no statistically significant difference in variance values for the compared series.

Excel file: exampl_9_17.xls

Example 9.18

Problem: In order to determine the values of repeatability, six independent series of measurements were performed for six standard solution samples. In each series, five repetitions were made.

Using the obtained measurement results, calculate repeatability for the analytical method.

Data: result series:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
1	2.54	5.12	7.14	10.2	14.2	17.3
2	2.67	5.16	7.15	10.9	14.8	17.8
3	2.43	5.24	7.34	11.3	13.9	17.2
4	2.65	5.34	7.09	10.2	14.3	17.0
5	2.34	5.02	7.34	10.1	14.4	17.5

SOLUTION: Because the levels of analyte concentrations in the investigated standard solutions samples are different, the calculations should use the values of *CV* and not *SD*.

The first step is to check the homogeneity of variances for individual series of results. Because series are equinumerous, one should apply the Hartley's F_{max} test (Section 1.8.6).

If variances are homogeneous, repeatability should be calculated as a mean value *CV* for the given series.

If variances are not homogeneous, one should reject the deviating value (series) and perform the calculations again.

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
Number of results – *n*	5	5	5	5	5	5
Standard deviation – *SD*	0.142	0.121	0.119	0.532	0.327	0.305
Coefficient of variation – *CV*, %	5.60	2.34	1.65	5.05	2.28	1.76
F_{max}				11.52		
F_{maxo} ($k = 6$, $f = 4$, $\alpha = 0.05$)				29.50		

Conclusion: Because $F_{max} < F_{maxo}$, there is no statistically significant difference in variance values for the compared series. It is possible to calculate repeatability as a mean value *CV* for the given series.

$CV_{repeatability}$	3.1%

Other possibilities are to calculate the CV of repeatability according to the following equation:

$$CV_{repeatability} = \sqrt{\frac{1}{k}\sum_{i=1}^{k} CV_i^2}$$

$CV_{repeatability}$	3.5%

In this case, the checking of homogeneity of variance is not necessary.

Excel file: exampl_9_18.xls

Example 9.19

Problem: To determine the values of repeatability and the intermediate precision of the analytical method, six independent series of measurements for the samples were performed for one standard solution. In each series, six repetitions were performed.

Using the obtained measurement results, calculate the values of repeatability and intermediate precision for the analytical method.

Data: result series, mg/L:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
1	101	103	111	100	103	103
2	104	106	107	102	102	108
3	103	102	104	101	106	102
4	101	105	102	117	103	107
5	100	109	110	115	107	105
6	102	104	105	103	104	103

SOLUTION: The first step is to check the homogeneity of the variances for individual series of results. Because series are equinumerous, one should apply the Hartley's F_{max} test.

If variances are homogeneous, repeatability should be calculated as a mean value CV for the given series.

If variances are not homogeneous, one should reject the deviating value (series) and perform the calculations again.

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
Number of results – *n*	6	6	6	6	6	6
Standard deviation – *SD*, *mg/L*	1.47	2.48	3.51	7.58	1.94	2.42
F_{max}				26.52		
F_{maxo} ($k = 6$, $f = 5$, $\alpha = 0.05$)				18.70		

Conclusion: Because $F_{max} > F_{maxo}$, there is a statistically significant difference in variance values for the compared series. The results from series 4 should be rejected due to the lack of homogeneity of variances, and calculations should be performed again.

Excel file: exampl_9_19a.xls

Data: result series:

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
1	101	103	111	–	103	103
2	104	106	107	–	102	108
3	103	102	104	–	106	102
4	101	105	102	–	103	107
5	100	109	110	–	107	105
6	102	104	105	–	104	103

	SERIES 1	SERIES 2	SERIES 3	SERIES 4	SERIES 5	SERIES 6
Number of results – *n*	6	6	6	0	6	6
Standard deviation – *SD*, *mg/L*	1.47	2.48	3.51	–	1.94	2.42
F_{max}				5.68		
F_{maxo} ($k = 5$, $f = 5$, $\alpha = 0.05$)				16.30		

Conclusion: Because $F_{max} < F_{maxo}$, there is no statistically significant difference in variance values for the compared series.

Repeatability was calculated as a mean of *SD* values for individual series.

Intermediate precision is *SD*, calculated using all the 30 results.

***SD* repeatability**	2.37	mg/L
***SD* intermediate precision**	2.75	mg/L

Excel file: exampl_9_19b.xls

9.2.7 Accuracy and Trueness

Accuracy is defined as closeness of agreement between a measured quantity value and a true quantity value of a measurand [26].

Trueness is the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value [26].

Analysis of these definitions shows that the hitherto existing notion of "accuracy" was replaced by the term "trueness", and the previously applied notion of "accuracy of a single measurement" is now simply "accuracy".

It is trueness that describes the conformity of results obtained using a given analytical method to real (expected) results. It is influenced mostly by the bias of the analytical method.

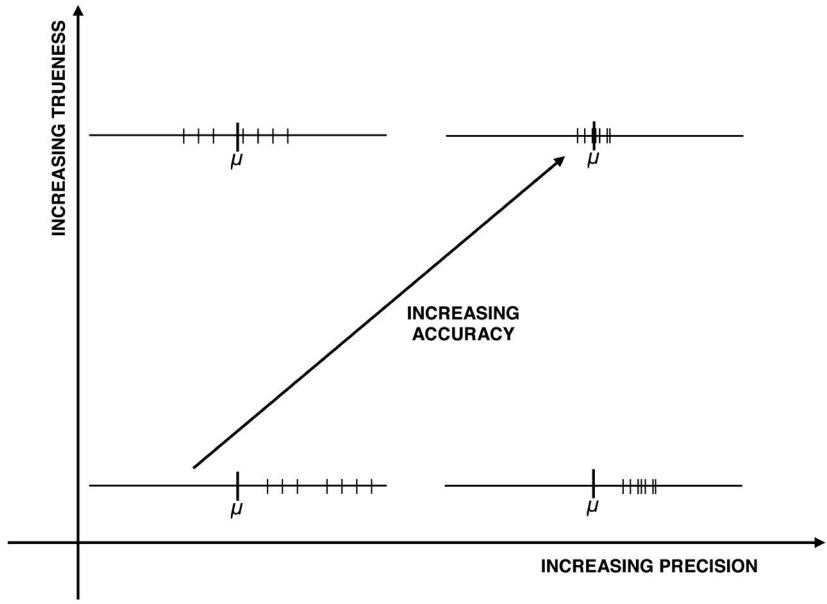


Figure 9.3 Relationships between trueness, precision and accuracy [4, 9].

Accuracy is a combination of trueness and precision. The truer and more precise the results obtained using a given method, the more accurate the result of a single measurement is. Relationships between trueness, precision, and accuracy are presented schematically in Figure 9.3 [4, 9].

Of course, other parameters such as linearity and sensitivity also influence the accuracy of an analytical method.

Trueness and accuracy can be determined using different approaches [33–39]:

- sample analysis of suitable certified reference materials,
- comparison of the obtained result with a result obtained using a reference (primary, definitive) method [40–42],
- standard addition method.

9.2.7.1 Measurement Errors The notion of accuracy is closely connected with the notion of errors [43]. Depending on the type of errors, their influence on measurements varies.

The value of a single measurement result may differ (and actually always differs) from the expected (real) value. The difference is due to

the occurrence of different errors [44]. There are three basic types of errors:

- gross errors,
- biases,
- random errors.

The influence of individual types of errors on a measurement result is presented schematically in Figure 9.4 [9].

With regard to the manner of presenting a determination result, one can distinguish:

- absolute error d_x which can be described by the dependence:

$$d_{x_i} = x_i - \mu_x \tag{9.12}$$

- relative error ε_x, described by the equation:

$$\varepsilon_{x_i} = \frac{d_{x_i}}{\mu_x} \tag{9.13}$$

With regard to the source of errors, one can distinguish:

- methodological errors,
- instrumental errors,
- human errors.

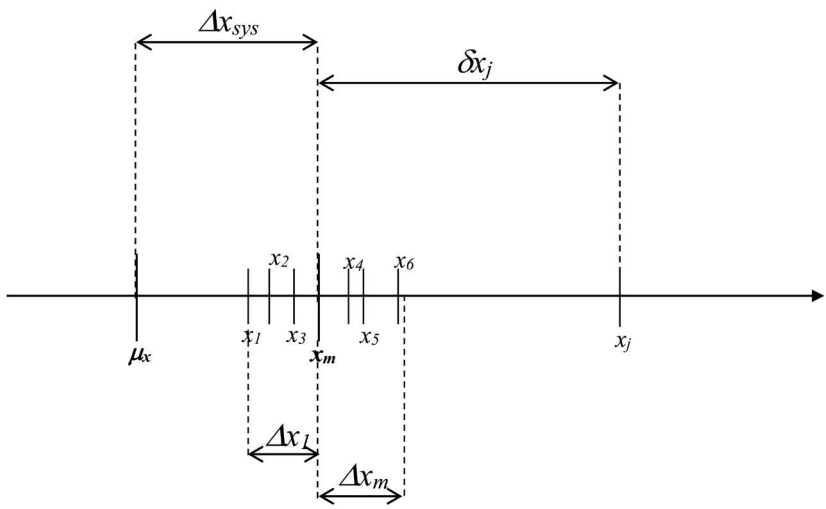


Figure 9.4 Influence of individual types of errors on a measurement result [9].

The total error of a single measurement result may be divided into three components, as described by the following equation [45]:

$$d_{x_i} = x_i - \mu_x = \Delta x_{sys} + \Delta x_i + \delta x_i \tag{9.14}$$

where:

d_{x_i} – total error of a measurement result,
x_i – value of a measurement result,
μ_x – expected value,
Δx_{sys} – bias,
Δx_i – random error,
δx_i – gross error.

For measurement series (at least three parallel analyte determinations in the same sample), there is a high probability of detecting a result(s) with a gross error.

Gross error is characterized by the following properties:

- it is the result of a single influence of a cause acting temporarily,
- it appears only in some measurements,
- it is a random variable – however, one with unknown distribution and unknown expected value,
- it is the easiest to detect, and therefore to eliminate,
- it assumes both positive and negative values (unlike bias),
- the cause of its occurrence can be, for example, a mistake in instrument reading or a mistake in calculations.

There are many known ways of detecting results with gross errors. Each of them is applied in certain specific conditions.

Methods of gross error determination are described in Chapter 1.

Figure 9.5 schematically presents the selection criteria for a suitable manner of action in detecting and rejecting results with gross errors, often described as "outliers" [9].

After eliminating results with gross errors, the trueness of the obtained final determination (most often the mean value of the measurement series) is influenced by biases and/or random errors.

The determination of biases is one way to determine the trueness of an analytical method.

Table 9.5 presents specific methods of bias determination [9].

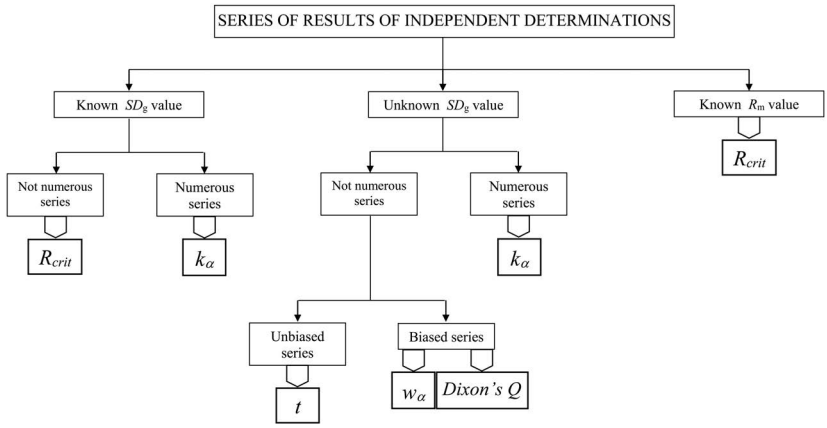


Figure 9.5 Selection criteria for a suitable manner of action in detecting and rejecting results with gross errors, often described as "outliers" [9].

Table 9.5 Basic Information Concerning Methods of Bias Determination [9]

BIAS TYPE	REQUIREMENTS	COURSE OF ACTION
Constant	Samples of two standards (reference materials) with different analyte content	Series determinations for two standard samples (reference material samples) with different analyte content, using the developed method. Constant bias a_{sys} is determined according to the formula: $$a_{sys} = \frac{\mu_{1x}x_{1m} - \mu_{2x}x_{2m}}{\mu_{1x} - \mu_{2x}} \quad (9.15)$$ where: μ_{1x}, μ_{2x} – the expected values for two standard samples, x_{1m}, x_{2m} – the mean values determined for standard samples.
Variable	Sample and sample with standard addition	Series determination with the use of the developed method for sample and sample with standard addition. The correction multiplier value is determined according to the formula: $$B = \frac{C_{st}}{x_{mCst} - x_m} \quad (9.16)$$ where: C_{st} – expected value increase of analyte concentration due to standard addition, x_m, x_{mCst} – mean values determined for sample and sample with standard addition. The value of variable bias is determined according to the equation: $$b_{sys} = \frac{1-B}{B} \quad (9.17)$$

(*Continued*)

Table 9.5 (*Continued*) Basic Information Concerning Methods of Bias Determination [9]

BIAS TYPE	REQUIREMENTS	COURSE OF ACTION
Variable	Samples of two standards (reference materials) Reference method	Two series of determination for two standard samples with the use of the reference method and the developed method. The correction multiplier value is determined according to the formula: $$B = \frac{x_{2m(ref)} - x_{1m(ref)}}{x_{2m} - x_{1m}} \quad (9.18)$$ where: $x_{1m(ref)}$, $x_{2m(ref)}$ – mean values determined for the first and second standard with using the reference method, x_{1m}, x_{2m} – mean values determined for the first and second standard with using the developed method. The value of variable bias is determined according to Equation (8.17).
Constant and variable	Series samples with different analyte content, Reference method	Series determination for samples with different analyte content with the use of the reference method and the developed method. The relationship between results obtained by the reference method (0Y-axis) and results obtained by the developed method (0X-axis) is determined. Regression parameters of the regression line $Y = b \cdot X + a$ are determined according to the Equations (1.63) and (1.64); The values of constant bias and variable bias are determined according to the formulas: a. constant bias: $$a = -a_{sys} b \quad (9.19)$$ therefore: $$a_{sys} = -\frac{a}{b} \quad (9.20)$$ b. variable bias: $$b = B \quad (9.21)$$ therefore: $$b_{sys} = \frac{1}{b} - 1 \quad (9.22)$$

A determination result (arithmetical mean of a series of parallel measurements) can only have a bias and random error according to the following dependence [45]:

$$d_{x_m} = x_m - \mu_x = \Delta x_{sys} + \Delta x_m \quad (9.23)$$

where:

d_{x_m} – total error of a determination result (arithmetical mean of the series of measurements),

x_m – mean value of the series of measurement results,
μ_x – expected value,
Δx_{sys} – bias,
Δx_m – random error.

If the determined bias refers to an analytical method, then with a large number of conducted measurements, the random error is negligibly small with relation to the bias, when $n \to \infty$, then $s \to 0$.

In this case, the following dependence is true [45]:

$$d_{x_{met}} = E\left(x_{met}\right) - \mu_x = \Delta x_{sys} \tag{9.24}$$

where:

$d_{x_{met}}$ – total error of a determination result for the applied analytical method,
$E(x_{met})$ – value of a determination obtained as a result of a given analytical method used (expected value for a given analytical method),
μ_x – expected value (real),
Δx_{sys} – bias.

In this way, the bias of an analytical method is determined. The occurrence of bias makes a given series of measurement (analytical method) results differ from the expected value by a constant value – hence, they are either overstated or understated.

One may differentiate between two types of bias:

- a constant bias, whose value is not relative to analyte concentration levels – a_{sys},
- a variable bias, whose value depends (most often linearly) on analyte concentration levels – $b_{sys}\,\mu_x$.

Bias is described by the dependence:

$$\Delta x_{sys} = a_{sys} + b_{sys}\mu_x \tag{9.25}$$

Assuming that the value of a random error is negligibly small compared to the bias value, one can present the following dependence:

$$x_m = \mu_x + \Delta x_{sys} = \mu_x + a_{sys} + b_{sys}\,\mu_x = a_{sys} + \left(1 + b_{sys}\right)\mu_x \tag{9.26}$$

Only after rejecting results with a gross error and determining biases (regarding their values and correcting the determination result), can a result have a random error? Its value influences the precision of the obtained results.

The trueness or accuracy can be determined using different techniques [37–39].

One of them is comparing the obtained measurement value with the value obtained resulting from a reference method for which the obtained results are treated as accurate. In this case, one can compare both results visually, but it is more metrologically correct to use Student's *t* test (Section 1.8.9) for the significance of differences between two results. Of course, this test can only be applied when the compared methods do not differ in a statistically significant manner with respect to precision (Snedecor's *F* test – Section 1.8.5).

However, when the result of the Snedecor's *F* test application is negative (standard deviations for the series of measurements obtained by the compared analytical methods differ statistically and significantly), one may use for "poor" (small) result series the "approximate test" of *Cochran's C* and *Cox* test (Section 1.8.10) or *Aspin* and *Welch* test (Section 1.8.11).

Another manner (most often applied) to determine the trueness or accuracy is the analysis of a reference material sample (or still better samples of the certified reference material) using the investigated analytical method.

According to the general definition, reference material is characterized by a constant and strictly defined analyte concentration and with a known concentration determination uncertainty [26]. Of course, it is not always possible to use reference material samples precisely satisfying given needs. In case of its inaccessibility, one should prepare a standard solution by adding a strictly specific quantity of analyte into the investigated sample and subject it to determination. In each case, however, one should perform independent determinations for a blank sample and correct the result for the sample with the known analyte concentration by the obtained measurement result.

In order to test if the obtained measurement value does not differ in a statistically significant manner from the certified value (expected value), one should apply Student's *t* test (Section 1.8.9).

An insignificant difference between the two obtained results may also be tested by using the method of calculating the ratio between the obtained results and uncertainties of their determination.

One determines the ratio of the obtained means (if the values did not differ between themselves, the ratio should be 1) and values of uncertainty for such a determined quantity. The inference is as follows: if the interval of a determined ratio ± the uncertainty of its determination ($R \pm U$) includes 1, one should infer that the compared mean values do not differ in a statistically significant manner.

Using obtained values, one should calculate the value of the R ratio according to the formula:

$$R = \frac{x_{det}}{x_{ref}} \tag{9.27}$$

and then the uncertainty U, using a dependence described by the equation:

$$U = k \frac{\sqrt{\left(u_{det}^2 + u_{ref}^2\right)}}{\left(\frac{x_{det} + x_{ref}}{2}\right)} \tag{9.28}$$

where:

U – expanded uncertainty for determined relation,

k – coverage factor whose value depends on the accepted level of probability (most often 95% for which $k = 2$).

There is also another manner based on the comparison of values calculated from the dependence which can be presented using the following expressions:

$$\left| x_{det} - x_{ref} \right| \tag{9.29}$$

$$2\sqrt{u_{(x_{det})}^2 + u_{(x_{ref})}^2} \tag{9.30}$$

where:

x_{det} – value of a determination result,

x_{ref} – reference value,

$u_{(x_{det})}$ – uncertainty of a determination result,

$u_{(x_{ref})}$ – uncertainty of a reference value.

Inference in this instance is as follows:

- if the inequality occurs:

$$\left|x_{det} - x_{ref}\right| < 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{ref})}}$$

  then the result is deemed to be in conformity with the reference value,
- when, however, the following dependence is true:

$$\left|x_{det} - x_{ref}\right| \geq 2\sqrt{u^2_{(x_{det})} + u^2_{(x_{ref})}}$$

  then the result is acknowledged to not be in conformity with the reference value.

This manner of inference is based on comparing differences between two results with the expanded uncertainty (for $k = 2$) calculated using the uncertainty for the compared values.

According to recommendations by the ICH [6], determining trueness should be carried out using at least nine parallel determinations at three different analyte concentration levels (at least three determinations per each level of concentration). The calculated trueness should be presented as the percentage of recovery of the expected value or as a difference between the mean and the expected value together with the given confidence interval.

EURACHEM [28] recommends 10 parallel determinations for a blank sample and the same number of determinations for reference material samples. The mean obtained for blank samples is then deduced from the mean obtained for the reference material, and so the corrected value is compared against the certified value.

It is also recommended to perform a series of measurements for the reference material using a so-called primary method, characterized by a null value of bias. In this case, the corrected mean obtained for the investigated method is compared with the one obtained by the primary method.

Example 9.20

Problem: In the given series of measurement results, check if there is a result with a gross error. Apply the confidence interval method, after the initial outlier rejection. Assume the value $\alpha = 0.05$.

Data: result series, mg/dm^3:

	DATA
1	8.8
2	7.8
3	9.2
4	9.5
5	6.3
6	8.2
7	9.1
8	8.8

α	0.05

SOLUTION:

x_{min}	6.3
x_{min+1}	7.8
x_{max}	9.5
x_{max-1}	9.2
t_{crit}	2.447

Initially, the result x_{min} was rejected.

x_m	8.77	mg/dm^3
SD	0.59	mg/dm^3

$$g = x_m \pm t_{crit}\sqrt{\frac{n}{n-2}}\,SD$$

g	8.77 ± 1.67 mg/dm^3
	(7.10 ÷ 10.44) mg/dm^3

Conclusion: The value x_{min} lies outside the determined confidence interval – hence, it has a gross error.

Excel file: exampl_9_20.xls

Example 9.21

Problem: Using the data from Example 9.20, apply the confidence interval method, without the initial outlier rejection. Assume the value $\alpha = 0.05$.

Data: result series, mg/dm^3:

	DATA
1	8.8
2	7.8
3	9.2
4	9.5
5	6.3
6	8.2
7	9.1
8	8.8

α	0.05

SOLUTION:

x_{min}	6.3
x_{max}	9.5
w_α	1.87

x_m	8.46	mg/dm^3
SD	1.0	mg/dm^3

$$g = x_m \pm w_\alpha \cdot SD$$

g	8.46 ± 1.93 mg/dm^3
	(6.53 ÷ 10.39) mg/dm^3

Conclusion: The value x_{min} lies outside the determined confidence interval – hence, it has a gross error. It should be rejected, and one the values of x_m and SD should be calculated for the new series of data.

x_m	8.77	mg/dm^3
SD	0.59	mg/dm^3

Excel file: exampl_9_21.xls

Example 9.22

Problem: Using the data from Example 9.20, apply the Dixon Q test. Assume the value $\alpha = 0.05$.

Data: result series, mg/dm^3:

	DATA
1	8.8
2	7.8
3	9.2
4	9.5
5	6.3
6	8.2
7	9.1
8	8.8

α	0.05

SOLUTION:

Number of results	8
Range – *R*	3.20
Q_1	0.469
Q_n	0.094
Q_{crit}	0.468

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Conclusion: Because $Q_1 > Q_{crit}$, the value x_{min} has a gross error. It should be rejected, and the values of x_m and SD should be calculated for the new series of data.

x_m	8.77	mg/dm^3
SD	0.59	mg/dm^3

Excel file: exampl_9_22.xls

Example 9.23

Problem: In a given series of measurement results, check if there are any results with a gross error. Apply the confidence interval method. Assume the value $\alpha = 0.05$.

Data: result series, ppm:

	DATA		DATA
1	13.2	**18**	13.2
2	13.7	**19**	13.3
3	13.9	**20**	13.7
4	14.1	**21**	13.7
5	13.4	**22**	13.8
6	13.2	**23**	13.2
7	13.4	**24**	14.1
8	13.7	**25**	14.2
9	14.2	**26**	13.9
10	11.3	**27**	13.2
11	13.4	**28**	13.6
12	13.2	**29**	13.4
13	13.8	**30**	13.7
14	14.2	**31**	14.1
15	14.2	**32**	14.0
16	15.8	**33**	13.8
17	15.4		

α	0.05

SOLUTION:

x_m	13.73	ppm
SD	0.72	ppm

k_α	1.65

$$g = x_m \pm k_\alpha \cdot SD$$

g	13.73 ± 1.19 ppm
	(12.53 ÷ 14.92) ppm

	DATA		DATA
1	13.2	**18**	13.2
2	13.7	**19**	13.3
3	13.9	**20**	13.7
4	14.1	**21**	13.7
5	13.4	**22**	13.8
6	13.2	**23**	13.2
7	13.4	**24**	14.1
8	13.7	**25**	14.2
9	14.2	**26**	13.9
10	~~11.3~~ Outlier	**27**	13.2
11	13.4	**28**	13.6
12	13.2	**29**	13.4
13	13.8	**30**	13.7
14	14.2	**31**	14.1
15	14.2	**32**	14.0
16	~~15.8~~ Outlier	**33**	13.8
17	~~15.4~~ Outlier		

Conclusion: Results 10th, 16th and 17th lie outside the determined confidence interval – hence have a gross error.

After their rejection, the values of x_m and *SD* were calculated again.

x_m	13.68	ppm
SD	0.36	ppm

Excel file: exampl_9_23.xls

Example 9.24

Problem: Check if there are results with a gross error in a given series of measurement results. The results of measurements were obtained using a method for which the standard deviation method had been determined.

Apply the critical range method. Assume the value $\alpha = 0.05$.

Data: result series, ppb:

	DATA
1	113
2	125
3	120
4	127
5	115
6	118
7	117
8	134
9	124

α	0.05
SD_g	4.5

SOLUTION:

x_{min}	113
x_{min+1}	115
x_{max}	134
x_{max-1}	127
z *(Table A3)*	4.39

R	21.0	ppb
R_{crit}	19.8	ppb

$$R_{crit} = z \cdot SD_g$$

Conclusion: Because $R > R_{crit}$, a result x_{max} is considered to be an outlier, and new calculations for the new series should be done.

Data (2): result series, ppb:

	DATA
1	113
2	125
3	120
4	127
5	115
6	118
7	117
8	–
9	124

SOLUTION (2):

x_{min}	113
x_{min+1}	115
x_{max}	127
x_{max-1}	125
z (*Table A.3*)	4.29

R	14.0	ppb
R_{crit}	19.3	ppb

Conclusion: Because $R < R_{crit}$, there are no more outliers in the series, and the values of x_m and SD could be calculated.

x_m	121	ppb
SD	6.65	ppb

Excel file: exampl_9_24.xls

Example 9.25

Problem: Check if there is a result with a gross error in a given series of measurement results. The results were obtained using a method for which a standard deviation had been determined before.

Apply the confidence interval method. Assume the value $\alpha = 0.05$.

Data: result series, ng/g:

	DATA		DATA
1	55.2	10	56.8
2	54.8	11	53.3
3	56.1	12	51.9
4	56.7	13	52.1
5	53.1	14	51.7
6	57.1	15	54.2
7	54.2	16	54.3
8	55.5	17	55.5
9	57.0		

α	0.05
SD_g	1.9

SOLUTION:

x_{min}	51.7
x_{min+1}	51.9
x_{max}	57.1
x_{max-1}	57.0
k_α	1.65

The result x_{min} was initially rejected.

The confidence interval value was calculated for the new series.

x_m	54.9	ng/g

$$g = x_m \pm k_\alpha \cdot SD_g \sqrt{\frac{n}{n-1}}$$

g	54.9 ± 3.2 ng/g
	(51.6 ÷ 58.1) ng/g

Conclusion: An initially rejected result x_{min} lies in the such determined confidence interval.

It has been included in the series, and the values of x_m and SD were calculated again.

x_m	54.7	ng/g
SD	1.8	ng/g

Excel file: exampl_9_25.xls

Example 9.26

Problem: Determinations were made for 25 samples, performing three parallel determinations per each sample. Using the data-obtained measurement results, check them for the occurrence of outliers.

Apply the critical range method. Assume the value $\alpha = 0.05$.

Data: result series, ppm:

SAMPLE	RESULT 1	RESULT 2	RESULT 3
1	3.01	3.33	3.35
2	3.11	3.04	3.13
3	3.65	3.45	3.41
4	3.23	3.45	3.12
5	3.22	3.13	3.33
6	3.28	3.41	3.62
7	3.45	3.12	3.04
8	3.65	3.07	3.45
9	3.01	3.08	3.99
10	3.14	3.52	3.88
11	3.11	3.71	3.12
12	3.65	3.74	3.07
13	3.23	3.32	3.04
14	3.67	3.22	3.2
15	3.98	3.11	3.44
16	3.56	3.41	3.49
17	3.33	3.49	3.82
18	3.11	3.51	3.72
19	3.23	3.82	3.23
20	3.41	3.01	3.67
21	3.21	3.01	3.98
22	3.48	3.37	3.56
23	3.6	3.62	3.33
24	3.62	3.08	3.62

α	0.05
z_α	1.96

SOLUTION:

SAMPLE	R_i	CONCLUSION	SAMPLE	R_i	CONCLUSION
1	0.34	OK	**13**	0.28	OK
2	0.09	OK	**14**	0.47	OK
3	0.24	OK	**15**	0.87	OK
4	0.33	OK	**16**	0.15	OK
5	0.20	OK	**17**	0.49	OK
6	0.34	OK	**18**	0.61	OK
7	0.41	OK	**19**	0.59	OK
8	0.58	OK	**20**	0.66	OK
9	0.98	Outlier	**21**	0.97	Outlier
10	0.74	OK	**22**	0.19	OK
11	0.60	OK	**23**	0.29	OK
12	0.67	OK	**24**	0.54	OK

R_m	0.49
R_{crit}	0.96

$$R_i = x_{\max_i} - x_{\min_i}$$

$$R_{crit} = z_\alpha \cdot R_m$$

Conclusion: For the series 9th and 21st, $R_i > R_{crit}$ results should be rejected as an outlier.

Excel file: exampl_9_26.xls

Example 9.27

Problem: Analyte concentrations were determined in two standard solution samples, with seven parallel determinations performed per sample. A second standard solution was obtained by double dilution of the first standard solution.

Using the obtained result series, determine the value of the constant bias a_{sys}.

Data: result series, ppm:

	RESULTS	
	SERIES 1	SERIES 2
1	10.01	5.33
2	10.11	5.04
3	10.07	5.11
4	10.23	5.45
5	10.22	5.13
6	10.28	5.41
7	10.23	5.12

x_{1st}	10.0
x_{2st}	5.0

SOLUTION:

x_{1m}	10.16
x_{2m}	5.23
k	2

$$k = \frac{x_{1st}}{x_{2st}}$$

$$a_{sys} = \frac{kx_{1m} - x_{2m}}{k-1}$$

a_{sys}	0.290	ppm

Excel file: exampl_9_27.xls

Example 9.28

Problem: Analyte concentrations were determined in a real sample and in a real sample with the standard addition. For each of the samples, six parallel measurements were made.

Using the data-obtained result series, determine the value of the variable bias b_{sys}. Using the calculated value of the correction multiplier, correct the values obtained for the real sample.

Data: result series, ppm:

	RESULTS	
	SERIES 1	SERIES 2
1	33.4	57.2
2	33.8	56.9
3	34.2	58.2
4	33.9	57.5
5	33.1	58.8
6	33.9	58.5

C_{st}	25.0

SOLUTION:

x_m	33.72
x_{mCst}	57.85

$$B = \frac{C_{st}}{x_{mC_{st}} - x_m}$$

$$b_{sys} = \frac{1-B}{B}$$

$$x_{m(corr)} = B \cdot x$$

B	1.036
b_{sys}	−0.035
$x_{m(corr)}$	34.93

Excel file: exampl_9_28.xls

Example 9.29

Problem: Analyte concentrations were determined in two real samples, using an investigated method and the reference method.

For each of the samples, eight parallel measurements were made, using both methods.

Using the obtained result series, determine the value of the variable bias b_{sys}. Using the calculated value of the correction multiplier, correct the values obtained using the validated method.

Data: result series, ppb:

	REFERENCE METHOD		VALIDATED METHOD	
	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
	x_{1ref}	x_{2ref}	x_1	x_2
1	746	945	765	967
2	740	947	772	980
3	753	956	758	978
4	758	960	768	984
5	743	948	783	974
6	750	955	749	984
7	746	960	777	975
8	755	966	769	988

SOLUTION:

$x_{1m(ref)}$	748.88
$x_{2m(ref)}$	954.63
x_{1m}	767.63
x_{2m}	978.75

$$B = \frac{x_{2m(ref)} - x_{1m(ref)}}{x_{2m} - x_{1m}}$$

$$b_{sys} = \frac{1 - B}{B}$$

$$x_{m(corr)} = B \cdot x$$

B	0.975
b_{sys}	0.026
$x_{1m(corr)}$	748.08
$x_{2m(corr)}$	953.83

Excel file: exampl_9_29.xls

Example 9.30

Problem: Analyte concentrations were determined in 15 real samples using the validated method and the reference method.

For each of the samples, three parallel measurements were made using each of the methods, and the mean values were presented.

Using the obtained data, determine the variable bias b_{sys} and the constant bias a_{sys}. Apply the linear regression method.

Data: result series, ppb:

	VALIDATED METHOD	REFERENCE METHOD
	x	x_{ref}
1	46.9	45.7
2	88.5	86.9
3	101	97.8
4	79.4	77.2
5	21.2	19.6
6	12.3	10.9
7	109	103
8	59.3	56.8
9	57.3	56.2
10	47.2	44.2
11	39.3	35.2
12	38.1	37.2
13	27.3	26.8
14	90.2	89.3
15	111	106

SOLUTION:

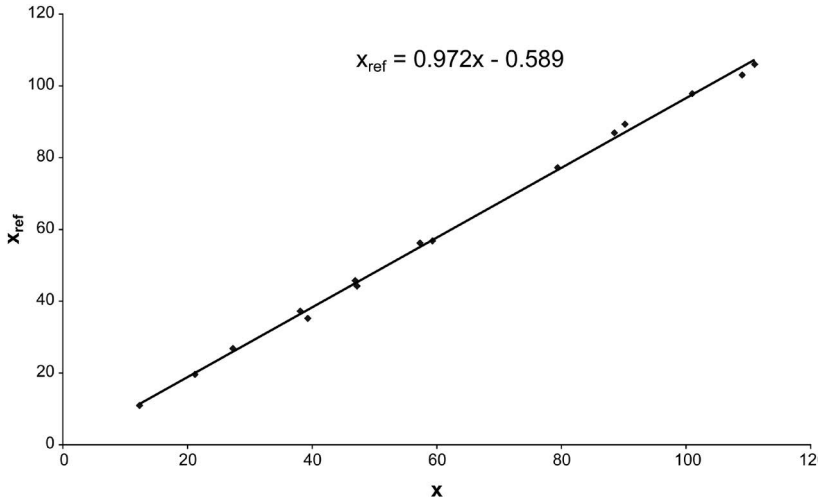
$$x_{ref} = b \cdot x + a$$

$$a_{sys} = -\frac{a}{b}$$

$$b_{sys} = \frac{1}{b} - 1$$

a	−0.589
b (B)	0.972
a_{sys}	0.607
b_{sys}	0.0292

Graph:



Excel file: exampl_9_30.xls

9.2.8 Robustness and Ruggedness

The robustness of a method is determined in order to find the influence of slight fluctuations of conditions in a given analytical method on the result of final determination. Robustness influences the manner of conducting measurements using a given analytical method. The greater the influence of slight changes in parameters of the measurement process on final determination results, the greater the attention one should pay to maintaining these parameters at a stable level. It is a parameter concerning changes in internal conditions [46, 47].

However, the ruggedness (flexibility) is a parameter describing the usefulness of a given analytical method in different conditions and can be estimated based on reproducibility [46, 47].

Similar to the reproducibility of an analytical method, its robustness and ruggedness are also determined in interlaboratory studies, although the influence of fluctuations from some measurement conditions (in a method subjected to validation) may be conducted in one laboratory (e.g. the influence of fluctuations in temperature, changes in purity and types of reagents, pH fluctuations, conditions of chromatographic isolations) [46, 47].

These parameters can be calculated based on a study of changes in the standard deviation of the measurement series using a given analytical method, and slightly fluctuating the parameters of the applied analytical method.

9.2.9 Uncertainty

Uncertainty is not considered a basic validation parameter, but it should be presented in the final method validation report. Based on the estimated uncertainty value, one can determine the usefulness of a given analytical method for a given determination. Determination of a combined uncertainty for an investigated analytical method (most often expressed as a percentage of the determined value) makes it possible to know the quality of results obtained with a given method. The exact characterization of this parameter, together with a description of its determination, is presented in Chapter 5.

Example 9.31

General problem: An analytical procedure was developed, indicating the content of total mercury content in samples of muscle tissue of great cormorant (*Phalacroxorac carbo*) with the use of atomic absorption spectroscopy (cold vapor technique). The validation process method was conducted, determining the appropriate validation parameters.

Problem 1: Determine the **selectivity** of the *CV-AAS* method.

SOLUTION: In the case of the cold vapor technique, mercury is released from the analyzed sample, and then (after an eventual reduction to atomic mercury) it is trapped on the gold bed as an amalgam. After this step, the amalgam is heated to 600°C, and the released atomic mercury is directed through the air stream to the absorption cell, in which an absorption measurement is conducted, with a wavelength of 253.7 nm, sent by a hollow mercury cathode lamp.

Conclusion: Such a measurement method guarantees high selectivity for indicating mercury for two reasons:

1. the amalgamation reaction is a selectivity reaction for mercury,
2. the absorption measurement is realized using a characteristic wavelength for mercury.

Problem 2: Based on measurement results for the series of standard solutions, determine the **linearity** of the method.

Data: results:

UNIT					
Content of Hg, ng	**20**	**40**	**60**	**80**	**100**
Signal	33.5	67.3	99.5	142.1	167.6
	34.1	66.4	98.3	137.8	175.2
	35.2	63.8	99.1	140.1	170.2
	32.8	68.1	100.2	136.2	169.3
	33.9	66.6	95.6	138.0	171.1

SOLUTION: Before constructing the calibration curve, the homogeneity of variation for the results of the series being analyzed should be checked. For this, Hartley's F_{max} test was applied, with a significance level of $\alpha = 0.05$.

Content of Hg	**20**	**40**	**60**	**80**	**100**
No. of results – *n*	5	5	5	5	5
Signal, mean	33.9	66.4	98.5	138.8	170.7
Standard deviation – SD	0.88	1.6	1.8	2.3	2.8
***CV*, %**	2.6	2.4	1.8	1.7	1.7
F_{max}			2.48		
F_{maxo}			25.20		

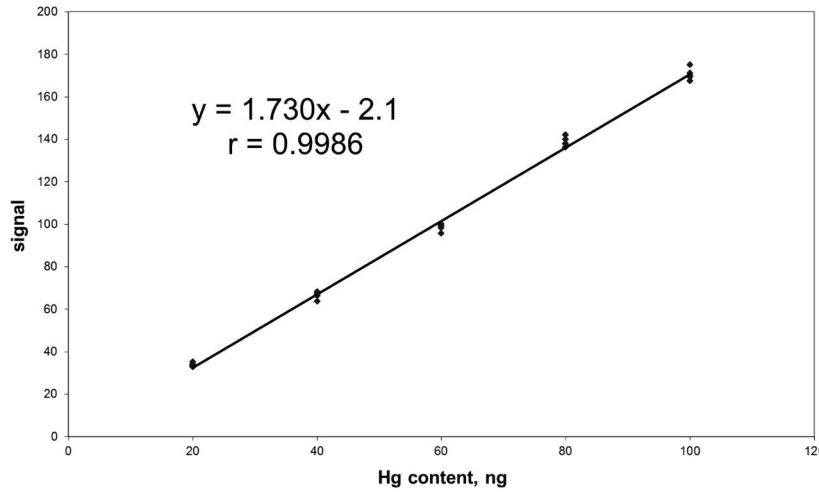
Conclusion: There are no statistically significant differences in variation values.

Excel file: exampl_9_31_1.xls

Due to no statistically significant differences in variation for the compared series, a calibration curve was constructed, and their regression parameters were determined.

n	25
Slope – *b*	1.730
Intercept – *a*	–2.1
Residual standard deviation – SD_{xy}	2.7
Standard deviation SD_b	0.019
Standard deviation SD_a	1.3
Regression coefficient – *r*	0.9986

Graph:



Excel file: exampl_9_31_2.xls

Conclusion: A high value of the regression coefficient, *r* with the fulfillment of the equal distribution of the standard in the range of the calibration line, requires a high linearity procedure.

Problem 3: Based on the series of measurement results for the standard solutions with the three lowest mercury content levels (20, 40 and 60 ng), determine the ***LOD*** value, the ***LOQ*** value and the **range**.

Additionally, check the correctness of the *LOD* determination.

SOLUTION:

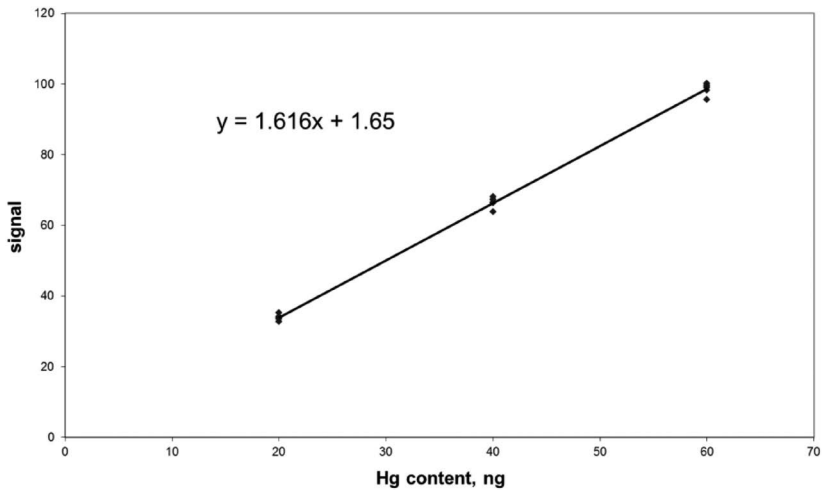
n	15
Slope – *b*	1.616
Intercept – *a*	1.65
Residual standard deviation – SD_{xy}	1.4
Standard deviation SD_b	0.023
Standard deviation SD_a	0.97
Regression coefficient – *r*	0.9987

The *LOD* value was determined using the equation:

$$LOD = \frac{3.3\,s}{b}$$

***LOD* (SD_{xy})**	2.9 ng
***LOD* (SD_a)**	2.0 ng
***LOD* (mean)**	2.5 ng

Graph:



The correctness of *LOD* determination was made according to the equations:

$$10 \cdot LOD > c_{\min}$$

$$LOD < c_{\min}$$

where:

c_{min} = 20 ng.

Conclusion: The determined *LOD* value is correct.

Based on the relationship:

$$LOQ = 3 \cdot LOD$$

The *LOQ* value was calculated to be:

$$LOQ = 7.4 \text{ ng}$$

While the range was presented as:

$$7.4 \div 100 \text{ ng}$$

Excel file: exampl_9_31_2.xls

Problem 4: Based on the series of results for the three real samples (lyophilized muscle tissue of great cormorant), calculate the **repeatability.**

Data: measurement results for individual samples:

	SAMPLE 1		
	SAMPLE MASS, mg	HG CONTENT, ng	HG CONCENTRATION, ppm
1	30.4	64.14	**2.11**
2	32.5	71.82	**2.21**
3	33.8	78.42	**2.32**
4	30.7	66.62	**2.17**
5	31.2	70.20	**2.25**
6	37.3	91.01	**2.44**
7	35.1	79.68	**2.27**
	Mean		**2.25**

	SAMPLE 2		
	SAMPLE MASS, mg	HG CONTENT, ng	HG CONCENTRATION, ppm
1	25.2	78.37	**3.11**
2	27.8	85.90	**3.09**
3	28.3	90.84	**3.21**
4	22.8	71.82	**3.15**
5	21.9	72.93	**3.33**
6	24.9	84.91	**3.41**
7	25.0	81.50	**3.26**
	Mean		**3.22**

	SAMPLE 3		
	SAMPLE MASS, mg	HG CONTENT, ng	HG CONCENTRATION, ppm
1	21.1	83.77	**3.97**
2	20.7	80.11	**3.87**
3	22.3	78.94	**3.54**
4	24.4	92.48	**3.79**
5	20.9	81.93	**3.92**
6	19.7	72.69	**3.69**
7	20.5	74.00	**3.61**
	Mean		**3.77**

SOLUTION: Before performing the calculation, in order to indicate precision, one should check whether in the measurement results series

there are no outliers. For this, the Dixon's Q test was applied (with a significance level of $\alpha = 0.05$).

	SAMPLE 1	SAMPLE 2	SAMPLE 3
No. of results – *n*	7	7	7
Range – *R*	0.33	0.32	0.43
Q_1	0.182	0.062	0.162
Q_n	0.363	0.250	0.116
Q_{crit}		0.507	

Conclusion: In the series of measurement results, there are no outliers.

Excel file: exampl_9_31_3.xls

Determinations were conducted for three different real samples; therefore, before calculating the repeatability value (as the mean of the coefficient variation for the results of the three series results), the homogeneity of the variation should be checked for the series of results to be analyzed. The Hartley's F_{max} test was applied with this aim (a significance level of $\alpha = 0.05$ was chosen).

	SAMPLE 1	SAMPLE 2	SAMPLE 3
No. of results – *n*	7	7	7
Standard deviation – *SD*	0.107	0.118	0.162
***CV*, %**	4.76	3.67	4.30
F_{max}		1.68	
F_{maxo}		8.38	

Conclusion: There are no statistically significant differences in variation values. The calculated repeatability value, however, can be calculated as a mean value from the coefficient of variation – *CV*, counted for three series:

$$CV_{repeatability} \qquad 4.24\%$$

Excel file: exampl_9_31_4.xls

Problem 5: Based on the results determined for certified reference material samples (BCR-463 – Tuna fish: total Hg and methylmercury), determine the **trueness** value (as a recovery value).

Data: results are given as (ng/mg):

	DATA
1	2.678
2	2.753
3	2.516
4	2.970
5	2.918

	Value	***U***	***k***
CRM	2.85	0.16	2

SOLUTION:

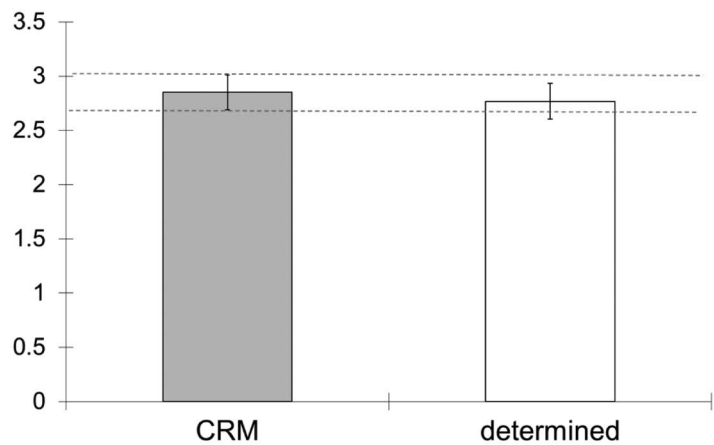
Mean	2.77
SD	0.184
U	0.16
%R	97.1
***U* (*k* = 2)**	8.2%

where the expanded uncertainty of the recovery value is calculated in accordance with the equation:

$$U = k\frac{\sqrt{\left(u_{CRM}^2 + u_{det}^2\right)}}{\left(\frac{x_{CRM} + x_{det}}{2}\right)}$$

Trueness = 97.1 ± 8.2%

Graph:



Conclusion: The results obtained with the use of the developed method are correct.

Excel file: exampl_9_31_5.xls

Problem 6: Estimate an **uncertainty** value for the determination results of the total mercury content in real samples, obtained with the use of the elaborated method.

SOLUTION: As the main components of the uncertainty budget, the following were recognized: the uncertainty value resulting from the calibration curve, the uncertainty value related to the unrepeatability of the measurement results, as well the uncertainty value from the indication of trueness.

The estimation of the combined uncertainty value was conducted using the relationship:

$$u_{smpl} = \sqrt{u_{cal}^2 + u_{rep}^2 + u_{true}^2}$$

where:

u_{smpl} – combined relative standard uncertainty for determined results for the real sample,

u_{cal} – relative standard uncertainty related to the calibration step,

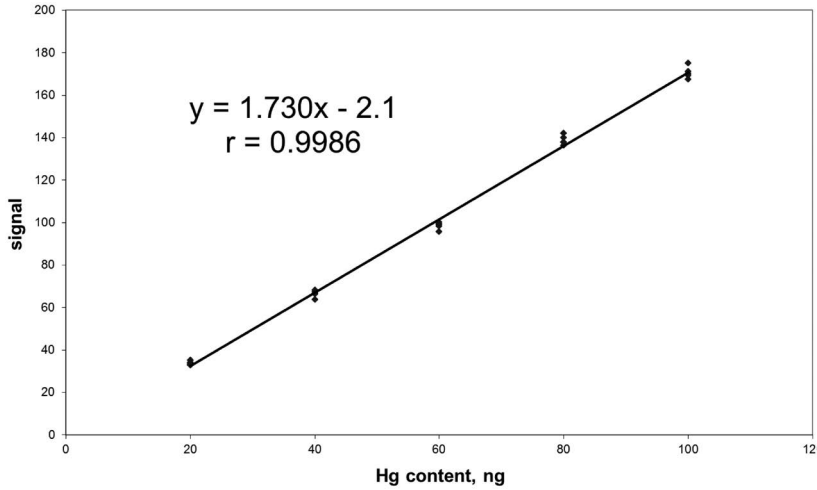
u_{rep} – relative standard uncertainty related to repeatability of measurement results,

u_{true} – relative standard uncertainty related to indicating trueness.

The determination of the standard uncertainty value related to the calibration step (preparation of the series of standard solutions, conducting measurements for the series of standard solutions, an approximation of measurement points of the calibration line using line regression) was conducted on the basis of the calibration parameters. Calculations were conducted for minimal weighted masses for each of the analyzed real samples.

	SAMPLE 1	SAMPLE 2	SAMPLE 3
No. of results – *n*	7	7	7
Minimum Hg content, ng	64.14	71.82	72.69
Hg, concentration, ppm	2.25	3.22	3.77
u_{cal}, %	1.1	0.96	0.96
u_{rep}, %	1.8	1.4	1.6
u_{true}, %		4.2	
u_{smpl}, %	4.7	4.5	4.6
u_{smpl}, ppm	0.11	0.15	0.17
U_{smpl} ($k = 2$), ppm	0.21	0.29	0.35
U_{smpl} ($k = 2$), %	9.4	9.1	9.3

Graph:



Conclusions: The estimated expanded uncertainty value for measurement results for real samples does not exceed 10% and allows for the notation of measurement results as follows:

Sample 1: 2.25 ± 0.21 ppm

Sample 2: 3.22 ± 0.29 ppm

Sample 3: 3.77 ± 0.35 ppm

Excel files: exampl_9_31_6.xls and exampl_9_31_7.xls

9.3 Conclusions

Validation of an analytical method should be finished with a final report containing [2, 9]:

- subject matter and the purpose of the analytical method (applicability range),
- metrological principles,
- type of the applied analyte(s) and matrix composition,
- list of all reagents, standards and reference materials used, together with precise specification (purity, quality, producer, and, in case of laboratory synthesis, a detailed description of this synthesis),

- description of the methods used for testing the purity of the substances used and the quality of standards,
- safety requirements,
- a plan describing the means of transferring the method from laboratory conditions to routine measurements,
- parameters of the method,
- a list of critical parameters whose slight fluctuations can significantly influence a final determination result – parameters resulting from determination of the analytical method's ruggedness,
- list of all types of laboratory instrumentation together with their characteristic features (dimensions, precision class, etc.), block schemes in case of complicated instrument kits,
- detailed description of the conditions for conducting the analytical method,
- description of statistical conduct together with the enclosed suitable equations and calculations,
- description of the method in order to inspect its quality in routine analyses,
- suitable figures and graphs, for example, chromatograms and calibration curves,
- conformity of the determined validation parameters with the assumed limits,
- the uncertainty of a measurement result,
- criteria that one should fulfill in revalidation,
- full name of the person who conducted the validation process,
- list of literature used,
- recapitulation and conclusions,
- confirmation and signature of the person responsible for the test and confirmation of the validation.

Example 9.32

Problem: Based on the validation parameters indicated for the analytical procedure in Example 9.31, create a validation report.

SOLUTION:
Seabirds are useful bioindicators of coastal and marine pollution. Marine birds spend a significant portion of their lives in coastal or

marine environments and are exposed to a wide range of chemicals because most occupy higher trophic levels, making them susceptible to bioaccumulation of pollutants.

Great cormorants (*Phalacrocorax carbo*) were used as bioindicators for mercury contamination, due to their specific feeding habits, wide geographical ranges and long life span.

The analytical procedure is intended for determining whole mercury content in muscle tissue samples from great cormorants.

Measurements of the content of total mercury will be performed using the cold vapor *AAS* technique.

A sample is thermally decomposed, mercury is further atomized, and free mercury vapor in the generated gas is collected by a mercury collection agent (gold-coated diatomite particle support) in the form of a gold amalgam. The mercury collection agent is then heated up to 600°C to release atomic mercury. The released mercury is detected using the cold atomic absorption method at a wavelength of 253.7 nm in the detector's absorption cell.

The analytical procedure pertains to the indication of total mercury content (after converting the total mercury content into an atomic form). Mercury content is determined in lyophilized muscle tissue of great cormorants.

During the analytical procedure, the following reagents are used:

- Mercury standard – MSHG – 100 ppm, concentration 100.48 ± 0.22 μg/mL in 3.3% HCl, Inorganic Ventures, Inc., USA,
- L-Cysteine, 98%, Nacalai Tesque, Inc., Kyoto, Japan,
- Additive B (activated alumina), Wako Pure Chemical Industries, Ltd., Japan,
- Additive M (sodium carbonate and calcium hydroxide), POCh, Poland,
- Nitric acid – suprapure, Merck, Germany
- Buffer solution pH 7.00 ± 0.05, POCh, Poland,
- CRM: BCR-463: Total and methyl mercury in tuna fish, 2.85 ± 0.16 μg/g, IRMM, Geel, Belgium,
- Deionized water.

PREPARATION OF STANDARD SOLUTIONS

There are various methods available for preparing standard solutions. Nippon Instrument Corporation obtained good results using L-cysteine. However, in this case, solution stability degrades with age or due to long storage in a warm place. Therefore, standard solutions should be kept in a cool and dark place.

Preparation of 0.001% L-Cysteine Solution

Measure 10 mg of L-cysteine and place it in a 1000 mL flask, then add water and 2 mL of guaranteed-reagent-grade concentrated nitric acid.

While ensuring uniformity of the contents in the flask by shaking it well, bring the total volume to 1000 mL by adding deionized water. For storage, keep in a cool and dark place.

Standard Solution Preparation

Take 1 mL of 100-ppm solution and dilute it to 10 mL with 0.001% L-cysteine solution. Now, a standard solution of 10-ppm has been prepared. By diluting in a similar manner, a standard solution of any concentration may be prepared. It should be noted that any mercury present in reagents or redistilled water should also be taken into consideration when a very dilute solution is prepared.

Any diluted solution, 100-ppm standard solution, and 10-ppm or less standard solution should be re-prepared after 1 year or 6 months have elapsed, respectively.

Before using a new volumetric flask, wash it with acid. In particular, when any solution of 1 ppm or less is prepared, carefully wash the flask with acid and ensure that its tap is thoroughly washed.

It is acceptable to use commercially available undiluted standard stock solutions (100 ppm or 1000 ppm) of mercury intended for atomic absorptiometry as $HgCl_2$. However, ensure that any mercury contained is in the form of $HgCl_2$. Some products contain $Hg(NO_3)_2$ as a mercury component. Since $Hg(NO_3)_2$ may react with L-cysteine and lose its function as a fixing agent, do not use standard undiluted $Hg(NO_3)_2$ solutions.

Mercury has toxic properties; therefore, during the preparation of standard solutions, it is advisable to adhere to procedure guidelines for these types of substances. The work should be conducted under a fume hood, using pipettes during the preparation of standard solutions. Protective attire should be worn: safety glasses, rubber gloves and lab coat.

Care should also be taken while working with the atomic absorption analyzer because of the high temperatures of some of its components, such as ovens heated up to 850°C.

For determining total mercury content in analyzed samples, an automatic mercury analyzer is utilized, MA-2000 from NIC (Japan). The Mercury/MA-2000 is a mercury analysis system that can measure mercury in liquid, solid and gas (optional parts required) samples.

Figure 9.32.1 Mercury MA-2000 analysis system.

As shown in Figure 9.32.1, the system consists of the mercury analyzer (MA-2), the sample changer (BC-1) and a personal computer (PC). Once samples are in position in BC-1, each of them in turn is automatically transferred to the analyzer to be measured. The PC reads the resulting measurements in the order that the various analyses, including statistical calculations, can be performed.

A block diagram of the apparatus is presented in Figure 9.32.2.

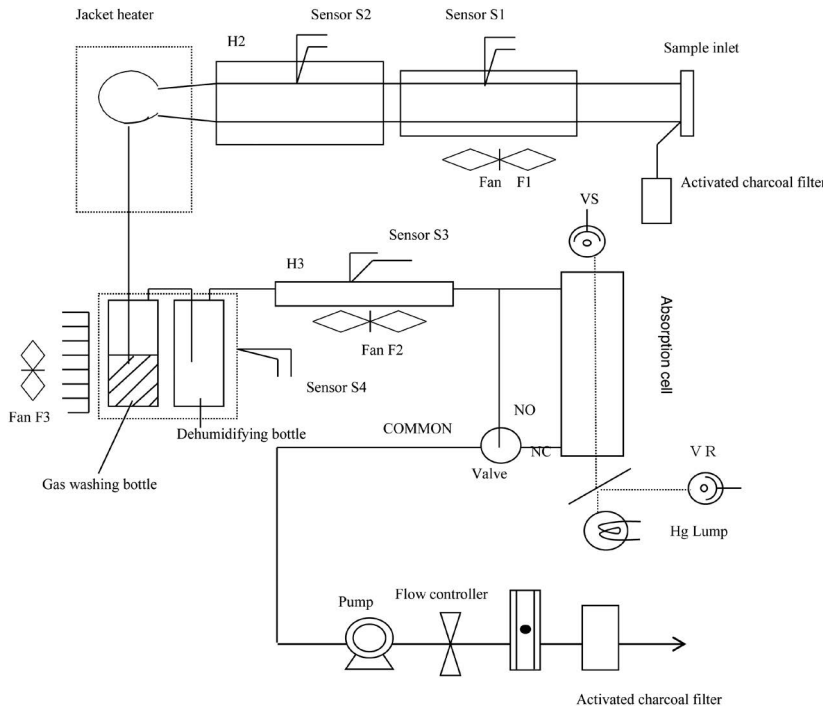


Figure 9.32.2 Schematic diagram of MA-2000.

ANALYTICAL PROCEDURE

Carefully separated bird tissues should be immediately deeply frozen, freeze-dried (lyophilized) and homogenized.

Homogenized samples should be stored in a refrigerator with a temperature of 0–6°C.

Homogenized samples should be directly weighed (10–50 ± 0.1 mg) into pre-cleaned combustion boats and automatically inserted into the Mercury/MA-2000 system (NIC – Japan).

To remove any interfering substances that are generated when thermally decomposing a sample, which would adversely affect measurements, gas washing is performed.

In addition, preheating the gold-coated diatomite particle support collection agent allows for the measurement to be done without the influence of any organic components, which would be physically absorbed to a certain extent, if not done so.

As a method of removing any substances that could interfere with the measurement, it is recommended that two kinds of additives be used: additive B (activated alumina) and additive M (sodium carbonate and calcium hydroxide). Before use, the additives should be subjected to a heat treatment in a heat treatment furnace at 750°C for at least three hours.

The sample boats which will be used should also be subjected to the same heat treatment.

The method for utilizing the additives is presented schematically in Figure 9.32.3.

CALIBRATION

Determine the calibration curve as a function of the peak surface area and the mercury content (Hg). Using an automatic pipette, dose at least five different volumes of the standard solution with a concentration of 1 ppm from the 20–100 μL section, which corresponds to 20–100 ng Hg.

For each mercury mass, repeat at least three times.

The minimal mass of the lyophilized tissue samples undergoing determination is limited on the one hand by the accuracy of the weight measurement, as well as the level of its homogeneity. Taking this into account, this value should not be less than 20 mg. However, the maximum sample mass is restricted by the maximum substance mass which can be introduced into the ceramic boat and consequently into the furnace. This value should not exceed 200 mg.

Taking this into account, the calibration curve corresponds to the range of Hg values in lyophilized tissue 0.1--5 ppm, or the values

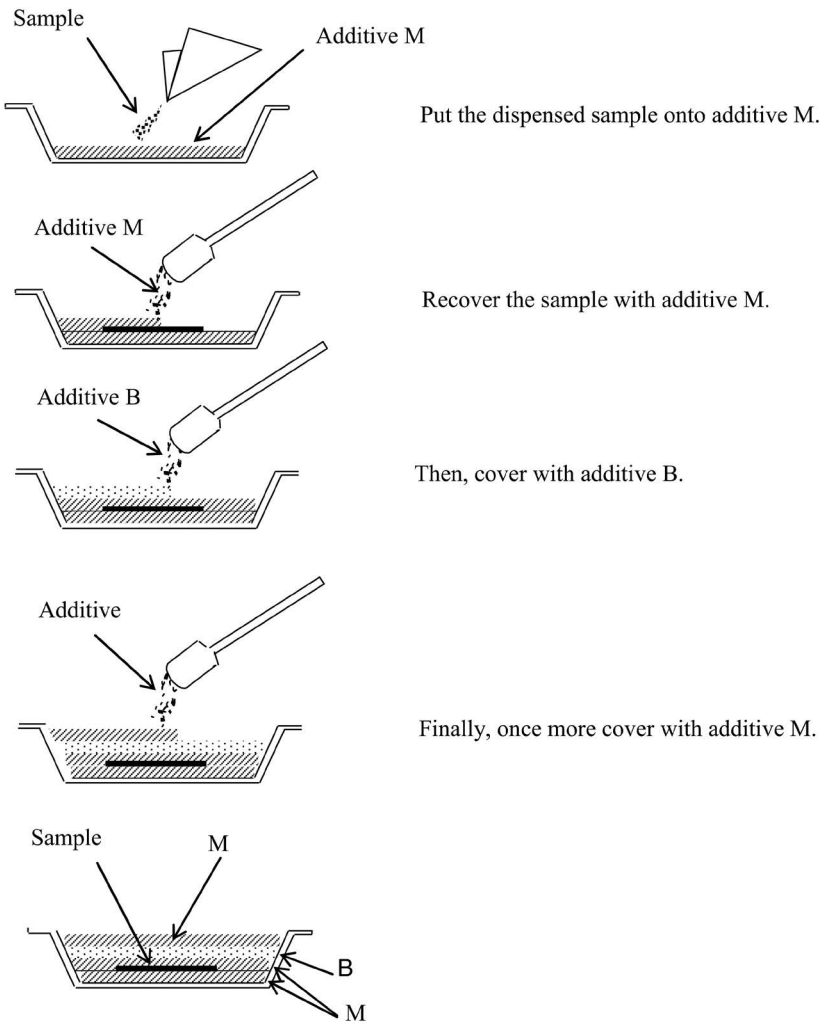


Figure 9.32.3 Method for using the additives.

corresponding to the section of the values which most often appear in the muscle tissue of great cormorants.

Draw the calibration curve and indicate the value of the regression parameters.

Compare these values with the determined values that are contained in the report.

The next steps of the analytical procedure are schematically presented in Figure 9.32.4.

During the analytical validation procedure, the values for the following parameters were indicated:

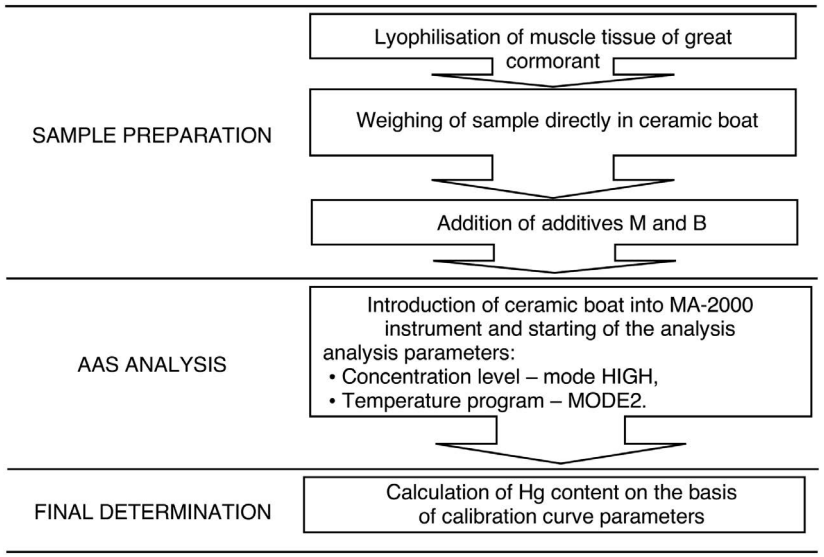


Figure 9.32.4 A schematic presentation of the analytical procedure for the determination of total mercury content in muscle tissue of great cormorant samples.

SELECTIVITY

Applying the measurement technique ensures high selectivity for indicating mercury for two reasons:

1. the amalgamation reaction is a selective reaction for mercury,
2. the absorption radiation measurement is realized for mercury's characteristic wavelength.

LINEARITY

A series of standard solutions were prepared with a mercury content of 20–100 ng. For each of the solutions, three independent measurements

Table 9.32.1 Calculated Regression Parameters for Linearity Determination

Number of results – *n*	25
Slope – *b*	1.730
Intercept – *a*	–2.1
Residual standard deviation – SD_{xy}	2.7
Standard deviation of the slope – SD_b	0.019
Standard deviation of the intercept – SD_a	1.3
Regression coefficient – *r*	0.9986

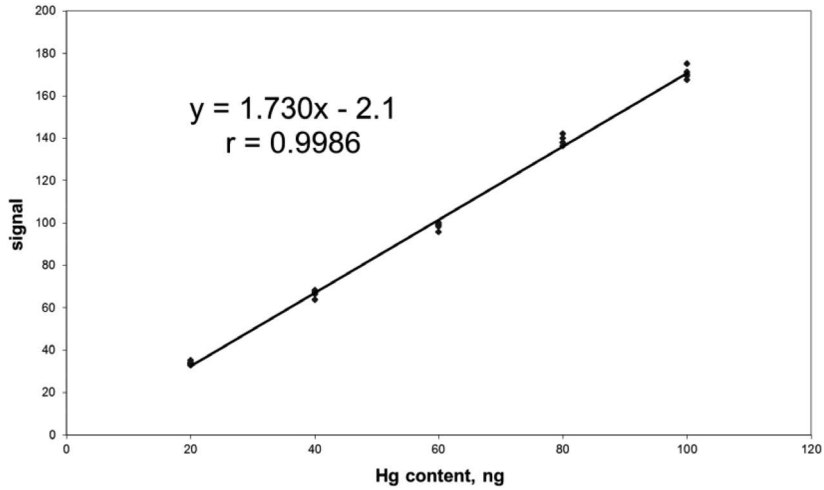


Figure 9.32.5 Calibration curve for linearity determination.

were conducted, and based on the obtained results, regression parameters were indicated and the calibration curve was determined. The obtained values are presented in Table 9.32.1, and the calibration curve is presented in Figure 9.32.5.

A high regression coefficient, r after fulfilling conditions for a "uniform" concentration distribution in terms of the calibration curve, commands a high linear procedure.

LIMIT OF DETECTION AND QUANTITATION

The *LOD* value is determined based on a series of measurement results for standard solution samples with the three lowest levels of mercury content (20, 40 and 60 ng). A calibration curve was outlined based on the obtained measurement results, parameters which determined *LOD* values, and the relationship:

$$LOD = \frac{3.3 \times SD}{b}$$

A calibration plot is presented in Figure 9.32.6.

The *LOD* value was deemed to be 2.5 ng, which, assuming the sample mass which underwent indication of an even 20 mg, corresponds to the mercury concentration in tissue samples of an even 0.12 ppm. However, the *LOQ* value was determined to be $LOQ = 3 \cdot LOD$, equaling 7.4 ng (assuming the mass of the 20 mg sample corresponds to a concentration of 0.37 ppm).

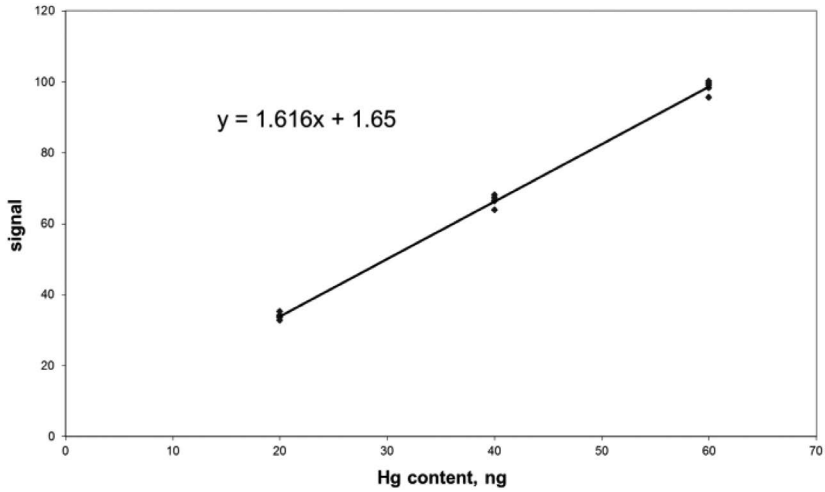


Figure 9.32.6 Calibration curve for *LOD* determination.

RANGE

The measurement range is a concentration range from the *LOQ* section, to a maximum standard solution concentration used for calibration. Therefore, it is equal to:

$$7.4 \div 100 \text{ ng}$$

which, assuming the mass of the sample which underwent indication is an even 20 mg, corresponds to a mercury concentration of:

$$0.37 \div 5.0 \text{ ppm}$$

REPEATABILITY

Repeatability is determined based on a series of measurement results for three real samples (muscle tissue of great cormorant after lyophilization). This value is determined as an average *CV* value for three series.

The determined repeatability value is equal to $CV_{\text{repeatability}} - 4.24\%$.

TRUENESS

The trueness value is determined based on determination results for certified reference material samples (BCR-463 – Tuna fish: total Hg and methylmercury) and is presented as a recovery value. A series of five independent determinations are conducted. The determined trueness value is equal to $97.1 \pm 8.2\%$.

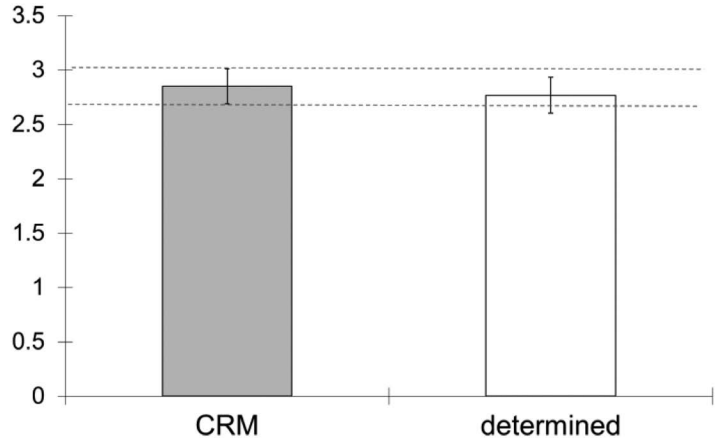


Figure 9.32.7 Comparison of the determined value with a certified Hg content value – trueness determination.

The determined trueness value is graphically presented in Figure 9.32.7.

UNCERTAINTY

The main components of the uncertainty budget were the uncertainty value resulting from the determination of the calibration curve, the uncertainty value related to the unrepeatability of measurement results, as well as the uncertainty value indicating trueness.

An estimation of the combined uncertainty value is conducted using the calculation:

$$u_{smpl} = \sqrt{u_{cal}^2 + u_{rep}^2 + u_{true}^2}$$

where:

u_{smpl} – combined relative standard uncertainty for determined results for the real sample,
u_{cal} – relative standard uncertainty related to the calibration step,
u_{rep} – relative standard uncertainty related to repeatability of measurement results,
u_{true} – relative standard uncertainty related to indicating trueness.

Determination of standard uncertainty related to the calibration step (preparation of a series of standard solutions, realization of measurements for the series of standard solutions, an approximation of measurement points of the calibration line with the use of linear

regression) is conducted based on calibration parameters. Calculations are conducted for minimal masses for each of the analyzed real samples.

The calculated uncertainty value for $k = 2$ equals 9.3% (as an average of the three samples).

During the revalidation process, attention should be paid to the stability of the calibration curve. The determined value parameters for the calibration curve should not differ by more than ± 5% in relation to values determined during the validation process (Table 9.32.1).

The consecutive parameter is trueness, indicated based on *CRM* determinations, as well as repeatability, whose value should not exceed $CV = 5\%$.

Conclusions

This analytical procedure fulfils the requirements for a procedure serving to determine whole mercury content in lyophilized tissue samples from muscle tissue of great cormorant.

The procedure is characterized by high selectivity, repeatability ($CV = 4.24\%$), trueness (recovery = 97.1% ± 8.2%), and therefore, high precision.

The results obtained using this method are characterized by low uncertainty (about 10%).

The estimated limit of detection value $LOD = 2.5$ ng of total mercury in the sample, assuming the minimal mass is an even 20 mg, corresponds to a concentration of 0.12 ppm and allows for the discovery of trace amounts of mercury in analyzed samples.

The validation process was conducted by Dr. Piotr Konieczka.
The validation report was checked and confirmed by Prof. Jacek Namieśnik.

Gdańsk, 20 June 2007

Bibliography

Ambrus Á., Doan V.V.N., Szenczi-Cseh J., Szemánné-Dobrik H., and Vásárhelyi, A. Quality control of pesticide residue measurements and evaluation of their results. *Molecules*, 28(3), 954, 2023, https://doi.org/10.3390/molecules28030954.

Analytical Methods Committee, Measurement of near zero concentration: recording and reporting results that fall close to or below the detection limit, *Analyst*, 126, 256–259, 2001.

Boening D.W., Ecological effects, transport and fate of Mercury: a general review, *Chemosphere*, 40, 1335–1351, 2000.

Downs S.G., Macleod C.L., and Lester J.N., Mercury in precipitation and its relation to bioaccumulation in fish: a literature review, *Water Air Soil Pollut*., 108, 149–187, 1998.

EURACHEM, *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*, Second Internet Edition, 2014, https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (access date 26.06.2024).

Houserová P., Hedbavny J., Matejcek D., Kracmar S., Sitko J., and Kuban V., Determination of total mercury in muscle, intestines, liver and kidney tissues of cormorant (*Phalacrocorax carbo*), great crested grebe (*Podiceps cristatus*) and Eurasian buzzard (*Buteo buteo*), *Vet. Med. - Czech*, 50(2), 61–68, 2005.

Houserová P., Kubáň V., Komar S., and Sitko J., Total mercury and mercury species in birds and fish in an aquatic ecosystem in the Czech Republic, *Environ. Pollut*. 145, 185–194, 2007.

Huber L., Validation of analytical methods, Agilent Technologies©, 2010, Publication Number 5990-5140EN.

Joint Committee for Guides in Metrology, *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)*, 2012

Johnston R.K., and Valente R.M., Specifying and evaluating analytical chemistry quality requirements for ecological risk assessment, Marine Environmental Support Office, Technical Memorandum 99-01, San Diego, 2000.

Kim E.Y., Saeki K., Tanabe S., Tanaka H., and Tatsukawa R., Specific accumulation of mercury and selenium in seabirds, *Environ. Pollut*. 94, 261–265, 1996.

Konieczka P., Misztal-Szkudlińska M., Namieśnik J., and Szefer P., Determination of total mercury in fish and cormorant using cold vapour atomic absorption spectrometry, *Pol. J. Environ. Stud*., 19, 931–936, 2010.

Mercury Analysis System, Mercury/MA-2000, Instruction Manual, Nippon Instruments Corporation, No. NIC-600-2009-04.

Misztal-Szkudlińska M., Szefer P., Konieczka P., and Namieśnik J., Biomagnification of mercury in trophic relation of Great Cormorant (*Phalacrocorax carbo*) and fish in the Vistula Lagoon, Poland, *Environ. Monit. Assess*., 176, 439–449, 2011.

Nam D.H., Anan Y., Ikemoto T., Okabe Y., Kim E.-Y, Subramanian A., Saeki K, and Tanabe S., Specific accumulation of 20 trace elements in great cormorants (*Phalacrocorax carbo*) from Japan, *Environ. Pollut*., 134, 503–514, 2005.

Ripp, J., Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits. [Madison, WI]: Wisconsin Department of Natural Resources, Laboratory Certification Program, 1996., 1996.

Saeki K., Okabe Y., Kim E.-Y., Tanabe S., Fukuda M., and Tatsukawa R., Mercury and cadmium in common cormorants (*Phalacrocorax carbo*), *Environ. Pollut.* 108, 249–255, 2000.

Teasdale A., Elder D., and Nims R. W. eds, ICH Quality Guidelines: An Implementation Guide, John Wiley & Sons, Inc, 2017.

Thompson M., Ellison S.L.R., and Wood R., Harmonized guidelines for single-laboratory validation of methods of analysis, *Pure Appl. Chem.*, 74, 835–855, 2002.

United States Pharmacopeia 47 – NF 42, 2024.

Vogelgesang J., and Hädrich J., Limits of detection, identification and determination: a statistical approach for practitioners, *Accred. Qual. Assur.*, 6, 242–255, 1998.

References

1. Danzer K., A closer look at analytical signals, *Anal. Bioanal. Chem.*, 380, 376–382, 2004.
2. Huber, L., Validation of Analytical Methods, 2010, http://www.chem.agilent.com/Library/primers/Public/5990-5140EN.pdf (access date 26.06.2024).
3. Ambrus Á., Doan V. V.N., Szenczi-Cseh J., Szemánné-Dobrik H., and Vásárhelyi, A., Quality control of pesticide residue measurements and evaluation of their results. *Molecules*, 28(3), 954, 2023, https://doi.org/10.3390/molecules28030954.
4. Konieczka P., The role of and place of method validation in the quality assurance and quality control (QA/QC) System, *Crit. Rev. Anal. Chem.*, 37, 173–190, 2007.
5. Traverniers I., De Loose M., and Van Bockstaele E., Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance, *Trends Anal. Chem.*, 23, 535–552, 2004.
6. Teasdale A., Elder D., and Nims R. W. eds, ICH Quality Guidelines: An Implementation Guide, John Wiley & Sons, Inc, 2017.
7. *European Pharmacopeia 11th edition*, 2022, online: https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition#{%22468369%22: (access date 26.06.2024).
8. United States Pharmacopeia 47 – NF 42, 2024.
9. Konieczka P., and Namieśnik J. eds., Kontrola i zapewnienie jakości wyników pomiarów analitycznych, WNT, Warsaw, 2017 (*in Polish*).
10. Vesseman J., Stefan R.I., Van Staden J. F., Danzer K., Lindner W., Burns D.T., Fajgelj A., and Müller H., Selectivity in analytical chemistry (IUPAC Recommendations 2001), *Pure Appl. Chem.*, 73, 1381–1386, 2001.
11. Valcárcel M., Gómez-Hens A., and Rubio S., Selectivity in analytical chemistry revisited, *Trends Anal. Chem.*, 20, 386–393, 2001.
12. Kapeller R., Quantifying selectivity: a statistical approach for chromatography, *Anal. Bioanal. Chem.*, 377, 1060–1070, 2003.

13. Danzer K., and Currie L.A., Guidelines for calibration in analytical chemistry, *Pure Appl. Chem.*, 70, 993–1014, 1998.
14. Thompson M., Ellison S.L.R., and Wood R., Harmonized guidelines for single-laboratory validation of methods of analysis, *Pure Appl. Chem.*, 74, 835–855, 2002.
15. Dobecki M. ed., Zapewnienie jakości analiz chemicznych, Instytut Medycyny Pracy im. Prof. J. Nofera, Łódź, 2004 (*in Polish*).
16. Ellison S.L.R., In defense of the correlation coefficient, *Accred. Qual. Assur.*, 11, 146–152, 2006.
17. González A.G., Herrador M.Á., Asuero A.G., and Sayago A., The correlation coefficient attacks again, *Accred. Qual. Assur.*, 11, 256–258, 2006.
18. Asuero A.G., Sayago A., and González A.G., The correlation coefficient: an overview, *Crit. Rev. Anal. Chem.*, 36, 41–59, 2006.
19. Van Loco J., Elskens M., Croux C., and Beernaert H., Linearity of calibration curves: use and misuse of the correlation coefficient, *Accred. Qual. Assur.*, 7, 281–285, 2002.
20. Hibbert D.B., Further comments on the (miss-)use of *r* for testing the linearity of calibration functions, *Accred. Qual. Assur.*, 10, 300–301, 2004.
21. Huber W., On the use of the correlation coefficient *r* for testing the linearity of calibration functions, *Accred. Qual. Assur.*, 9, 726–726, 2004.
22. De Souza S.V.C., and Junqueira R.G., A procedure to assess linearity by ordinary least squares method, *Anal. Chim. Acta*, 552, 25–35, 2005.
23. Mulholland M., and Hibbert D.B., Linearity and the limitations of least squares calibration, *J. Chromatogr. A*, 762, 73–82, 1997.
24. Michulec M., and Wardencki W., Development of headspace solid-phase microextraction–gas chromatography method for the determination of solvent residues in edible oils and pharmaceuticals, *J. Chromatogr. A*, 1071, 119–124, 2005.
25. Michulec M., and Wardencki W., The application of single drop extraction technique for chromatographic determination of solvent residues in edible oils and pharmaceutical products, *Chromatographia*, 64, 191–197, 2006.
26. Joint Committee for Guides in Metrology, *International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM)*, JCGM 200:2012
27. Konieczka P., Sposoby wyznaczania granicy wykrywalności i oznaczalności, *Chem. Inż. Ekol.*, 10, 639–654, 2003, *(in Polish)*.
28. EURACHEM, *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*, Second Internet Edition, 2014, https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (access date 26.06.2024).
29. Analytical Detection Limit Guidance, Wisconsin Department of Natural Resources, 1996. http://www.allaboutair.cn/uploads/soft/171120/DL.pdf

30. Geiß S., and Einax J.W., Comparison of detection limits in environmental analysis – is it possible? An approach on quality assurance in the lower working range by verification, *Fresenius J. Anal. Chem*., 370, 673–678, 2001.
31. Namieśnik J., and Górecki T., Quality of analytical results, *Rev. Roum. Chim*., 46, 953–962, 2001.
32. Świtaj-Zawadka A., Konieczka P., Przyk E., and Namieśnik J., Calibration in metrological approach, *Anal. Lett*., 38, 353–376, 2005.
33. ISO 5725-1:2023. Accuracy (trueness and precision) of measurement methods and results – part 1: general principles and definitions.
34. ISO 5725-2:2019. Accuracy (trueness and precision) of measurement methods and results – part 2: basic method for the determination of repeatability and reproducibility of a standard measurement method.
35. ISO 5725-3: 2023. Accuracy (trueness and precision) of measurement methods and results – part 3: intermediate precision and an alternative designs for collaborative studies.
36. ISO 5725-4:2020. Accuracy (trueness and precision) of measurement methods and results – part 4: basic method for the determination of the trueness of a standard measurement method.
37. ISO 5725-5:1998. Accuracy (trueness and precision) of measurement methods and results – part 5: alternative methods for the determination of the precision of a standard measurement method.
38. ISO 5725-6:1994. Accuracy (trueness and precision) of measurement methods and results – part 6: use in practice accuracy values.
39. De Castro M., Bolfarine H., Galea-Rojas M., and De Castilho M.V., An exact test for analytical bias detection, *Anal. Chim. Acta*, 538, 375–381, 2005.
40. Hulanicki A., Absolute methods in analytical chemistry, *Pure Appl. Chem*., 67(11), 1905–1911, 1995.
41. Dybczyński R., Danko B., Polkowska-Motrenko H., and Samczyński Z., RNAA in metrology: a highly accurate (definitive) method, *Talanta*, 71, 529–536, 2001.
42. Richter W., Primary methods of measurement in chemical analysis, *Accred. Qual. Assur*., 2, 354–359, 1997.
43. Hibbert D.B., Systematic errors in analytical chemistry, *J. Chromatogr. A*., 1158, 25–32, 2007.
44. Maj-Żurowska M., Pyrzyńska K., Wagner B., Palińska-Saadi A., Współczesna chemia analityczna, PWN, PZWL Warsaw, 2022 (*in Polish*).
45. Kozłowski E., Statystyczne kryteria oceny wyników i metod analitycznych in Bobrański B. (ed.): Analiza ilościowa związków organicznych, PWN, Warsaw, 1979 (in Polish).
46. Cuadros-Rodríguez L., Romero R., and Bosque-Sendra J.M., The role of the robustness/ruggedness and inertia studies in research and development of analytical processes, *Crit. Rev. Anal. Chem*., 35, 57–69, 2005.
47. Dejaegher B., and Vander Heyden Y., Ruggedness and robustness testing, *J. Chromatogr. A*., 1158, 138–157, 2007.

10
Method Equivalence

10.1 Introduction

Equivalent method is defined as a measurement method other than the reference method for the measurement for which equivalence has been demonstrated.

In cases where it is not possible to use the reference method (norm) in the laboratory – for example, due to the lack of a suitable apparatus – it is necessary to document method equivalence. This is a confirmation that the results obtained by the method used in the laboratory agreed with the reference method. Method equivalence shall also apply in the case where norm method is more expensive and time-consuming than that which is used in the laboratory. Method equivalence is the answer to the question whether the parameters of the test methods and reference methods are not significantly different and statistically significant. This is particularly required in the case of non-regulated method, to prove no statistically significant differences in the results, for example, in the course of the accreditation process.

10.2 Ways of Equivalence Demonstration

Validation parameters, as described in Chapter 8, are calculated based on the values of the statistical parameters such as mean (trueness, accuracy) and/or the standard deviation (linearity, limit of detection [LOD], limit of quantitation [LOQ], precision, robustness, ruggedness).

For this reason, the demonstration of method equivalence is the first and foremost indication of compliance obtained using the exanimated method and the reference method values of mean and standard deviation. Depending on the type of data sets and strategy of equivalence method demonstration, there are three basic ways of proceeding.

10.2.1 Difference Testing [1–4]

Difference tests have been widely used to answer questions about whether a disparity has been successfully addressed; however, these tests are subject to well-known limitations, and the results are sometimes misinterpreted. In tests of difference, analysts test the null hypothesis that the set of data under consideration does not differ. In difference testing, the null hypothesis is "no difference". If the analysis reveals a statistically significant difference between groups, the null hypothesis of no difference is rejected. However, if the analysis does not reveal a statistically significant difference between groups, the null hypothesis must stand – it cannot be rejected.

As statistical tests Student's t (for mean comparison) and Snedecor's F (for standard deviation comparison) are mainly used.

For the comparison of more than two sets of data, analysis of variance (ANOVA) is often used.

By using a difference test is the answer for the question: Is it likely that no difference exists between two sets of results?

Example 10.1

Problem: Determine the equivalence of the examined method based on the given series of measurement results, results of determination for CRM and the precision of reference method. Assume the value $\alpha = 0.05$.

Data: result series, mg/dm^3:

	DATA
1	12.56
2	12.75
3	13.11
4	12.31
5	12.98
6	13.06

CRM, mg/dm^3

x_{CRM}	10.56	x_{det}	11.6
U_{CRM}	0.65	U_{det}	1.5
k	2	k	2

Precision of reference method: $CV_o = 2.0\%$

SOLUTION:

1. Checking for outlier using Dixon-Q test.

No. of results – n	6
Range – R	0.80
Q_1	0.313
Q_n	0.062
Q_{crit}	0.560

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, there is no outlier in the results series. The calculated values of x_m, SD and CV are:

x_m	12.80	mg/dm^3
SD	0.32	mg/dm^3
CV	2.5	%

2. Check (at the level of significance $\alpha = 0.05$) if the calculated CV differs statistically significantly from the CV_o for reference method. Apply the χ^2 test.

Number of results – n	6
CV	2.5
χ^2	9.09
χ^2_{crit} ($f = 5$, $\alpha = 0.05$)	11.07

The calculation was performed using Equation 1.29 – Chapter 1, Subsection 1.8.4.

Because $\chi^2 < \chi^2_{crit}$, there is no statistically significant difference in CV values (precision). The exanimated method does not differ statistically significant in precision.

3. Compare the result obtained for CRM with certified value, calculate trueness as a recovery value for $k = 2$.

%R	110%
$U(k = 2)_{\%R}$	15%

$$\%R = \frac{x_{det}}{x_{CRM}} \cdot 100\%$$

$$U = k \cdot \frac{\sqrt{\left(u^2_{(x_{det})} + u^2_{(x_{CRM})}\right)}}{\left(\frac{x_{det} + x_{CRM}}{2}\right)}$$

A value of 100% is in the range of calculated trueness value.

Conclusion: The results obtained by the investigated method do not differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_1.xls

Example 10.2

Problem: Determine the equivalence of the examined method based on the given series of measurement results, obtained by the examined method and the reference method.

Data: result series, mg/dm^3:

	DATA	
	EXAMINATED METHOD	REFERENCE METHOD
1	12.56	13.07
2	12.75	13.23
3	13.11	13.10
4	12.31	12.98
5	12.98	13.33
6	13.06	13.06

SOLUTION:

1. Checking for outliers using Dixon-*Q* test.

	EXAMINATED METHOD	REFERENCE METHOD
No. of results – n	6	6
Range – R	0.80	0.35
Q_1	0.313	0.229
Q_n	0.062	0.286
Q_{crit}	0.560	0.560

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, for both series, there are no outliers in the results series. The calculated values of x_m, SD and CV are:

	EXAMINATED METHOD	REFERENCE METHOD	
x_m	12.80	13.13	mg/dm^3
SD	0.32	0.13	mg/dm^3
CV	2.5	1.0	%

2. Check (at the level of significance $\alpha = 0.05$) if the standard deviation values for both the series are statistically significantly different.
   Apply the Snedecor's F test.

F	6.06
F_{crit}	5.05

The calculation was performed using Equation 1.30 – Chapter 1, Subsection 1.8.5.

Because $F > F_{crit}$, there is a statistically significant difference in variance values for the compared series, and the series differs in precision.

Conclusion: The results obtained by the investigated method differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_2.xls

Example 10.3

Problem: Determine the equivalence of the examined method based on the given series of measurement results obtained by the examined method and the reference method.

Data: result series, mg/dm^3:

	DATA	
	EXAMINATED METHOD	REFERENCE METHOD
1	12.56	13.07
2	12.75	13.27
3	13.11	13.10
4	12.31	12.91
5	12.98	13.33
6	13.06	13.06

SOLUTION:

1. Checking for outliers using Dixon-Q test.

	EXAMINATED METHOD	REFERENCE METHOD
No. of results – n	6	6
Range – R	0.80	0.42
Q_1	0.313	0.357
Q_n	0.062	0.143
Q_{crit}	0.560	0.560

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, for both series, there are no outliers in the results series. The calculated values of x_m, SD and CV are:

	EXAMINATED METHOD	REFERENCE METHOD	
x_m	12.80	13.12	mg/dm^3
SD	0.32	0.15	mg/dm^3
CV	2.5	1.2	%

2. Check (at the level of significance $\alpha = 0.05$) if the standard deviation values for both the series are statistically significantly different.
Apply the Snedecor's F test.

F	4.24
F_{crit}	5.05

The calculation was performed using Equation 1.30 – Chapter 1, Subsection 1.8.5.

Because $F < F_{crit}$, there is no statistically significant difference in variance values for the compared series, and the series does not differ in precision.

3. Check (at the level of significance $\alpha = 0.05$) if the means for both the series are statistically significantly different.
Apply the Student's t test.

t	2.296
t_{crit}	2.228

The calculation was performed using Equation 1.38 – Chapter 1, Subsection 1.8.9.

Because $t > t_{crit}$, there is a statistically significant difference in the means for the compared series, and the series differs in accuracy.

Conclusion: The results obtained by the investigated method differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_3.xls

Example 10.4

Problem: Determine the equivalence of the examined method based on the given series of measurement results obtained by the examined method and the reference method.

Data: result series, mg/dm^3:

	DATA	
	EXAMINED METHOD	REFERENCE METHOD
1	12.56	13.07
2	12.75	13.27
3	13.11	13.10
4	12.31	12.91
5	12.98	13.74
6	13.06	13.06

SOLUTION:

1. Checking for outliers using Dixon-Q test.

	EXAMINED METHOD	REFERENCE METHOD
No. of results – n	6	6
Range – R	0.80	0.83
Q_1	0.313	0.181
Q_n	0.062	0.566
Q_{crit}	0.560	0.560

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because $Q_n > Q_{crit}$, for reference method series, there is an outlier in the series. So, the values of x_m, SD and CV were calculated for six results in the examined method series and for five results in the reference method:

	EXAMINED METHOD	REFERENCE METHOD	
x_m	12.80	13.08	mg/dm^3
SD	0.32	0.13	mg/dm^3
CV	2.5	1.0	%
n	6	5	

2. Check (at the level of significance $\alpha = 0.05$) if the standard deviation values for both the series are statistically significantly different.
   Apply the Snedecor's F test.

F	6.02
F_{crit}	6.26

   The calculation was performed using Equation 1.30 – Chapter 1, Section 1.8.5.

   Because $F < F_{crit}$, there is no statistically significant difference in variance values for the compared series, and the series does not differ in precision.
3. Check (at the level of significance $\alpha = 0.05$) if the means for both the series are statistically significantly different.
   Apply the Student's t test.

t	1.897
t_{crit}	2.262

   The calculation was performed using Equation 1.38 – Chapter 1, Subsection 1.8.9.

   Because $t < t_{crit}$, there is no statistically significant difference in means for the compared series, and the series does not differ in accuracy.

Conclusion: The results obtained by the investigated method do not differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_4.xls

10.2.2 Equivalence Testing [1–5]

In equivalence testing, the null hypothesis is formulated so that the statistical test is proof of similarity; it states that the groups differ by more than a tolerably small amount.

In equivalence testing, the null hypothesis is "a difference of certain limit or more". In equivalence testing, the null hypothesis states the difference among group means is greater than some minimal difference representing practical equivalence. The alternative hypothesis is that the difference is not greater than this specified minimum difference.

Equivalence testing is used when one wants assurance that the means do not differ too much. In other words, the means are

practically equivalent. A threshold difference acceptance criterion is set by the analyst for each parameter under the test. The means are considered equivalent if the difference in the two groups is significantly lower than the upper practical limit and significantly higher than the lower practical limit.

So, equivalence tests can be used for study:

- comparison to a reference standard or target,
- comparison between two series,
- comparison of slopes for stability,
- comparison of intercepts.

If one wants to determine equivalence, a more appropriate statistical question to ask is perhaps: is there an unacceptable difference between two sets of results?

If Student's t test has to be apply for that purpose, the modified equation has to be used:

$$t = \frac{\left| x_{m_{ref}} - x_{m_{exam}} \right| - \Delta\% \cdot x_{m_{ref}}}{\sqrt{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}} \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}} \qquad (10.1)$$

where:

Δ [in %] – limit of differences between compared values.

Example 10.5

Problem: Determine the equivalence of the examined method based on the given series of measurement results obtained by the examined method and the reference method. In the case of means comparison, take into account a limit of difference equal to ±3%.

Data: result series, mg/dm^3:

	DATA	
	EXAMINED METHOD	REFERENCE METHOD
1	12.56	13.07
2	12.75	13.27
3	13.11	13.45
4	12.31	13.14
5	12.98	13.3
6	13.06	13.06

SOLUTION:

1. Checking for outliers using Dixon-Q test.

	EXAMINED METHOD	REFERENCE METHOD
No. of results – n	6	6
Range – R	0.80	0.39
Q_1	0.313	0.026
Q_n	0.062	0.308
Q_{crit}	0.560	0.560

The calculation was performed using Equation 1.25 – Chapter 1, Subsection 1.8.3.

Because Q_1 and $Q_n < Q_{crit}$, for both series, there are no outliers in the results series. The calculated values of x_m, SD and CV are:

	EXAMINED METHOD	REFERENCE METHOD	
x_m	12.80	13.22	mg/dm^3
SD	0.32	0.16	mg/dm^3
CV	2.5	1.2	%

2. Check (at the level of significance $\alpha = 0.05$) if the standard deviation values for both the series are statistically significantly different.
Apply the Snedecor's F test.

F	4.07
F_{crit}	5.05

The calculation was performed using Equation 1.30 – Chapter 1, Subsection 1.8.5.

Because $F < F_{crit}$, there is no statistically significant difference in variance values for the compared series, and the series does not differ in precision.

3. Check (at the level of significance $\alpha = 0.05$) if the means for both the series are statistically significantly different. Take into account a limit of difference equal to ±3%.
Apply the Student's t test.

$$t = \frac{\left| x_{m_{ref}} - x_{m_{exam}} \right| - 3\% \cdot x_{m_{ref}}}{\sqrt{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}} \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2}}$$

t	0.198
t_{crit}	2.262

Because $t < t_{crit}$, there is no statistically significant difference in the means for the compared series, and the series does not differ in accuracy.

Conclusion: The results obtained by the investigated method do not differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_5.xls

10.2.3 Regression Analysis Testing

In the case when it is possible to have sets of data for different contents obtained by using both methods, it is recommended to apply regression analysis. The way of proceeding is than to calculate regression line parameters and, by using Student's t test, compare an obtained values with expected ones.

Example 10.6

Problem: Determine the equivalence of the examined method based on the given series of measurement results for real samples obtained by the examined method and the reference method. Apply the linear regression method.

Data: result series, ppb:

	EXAMINED METHOD	REFERENCE METHOD
	y	x
1	46.9	45.7
2	88.5	86.9
3	101.0	97.8
4	79.4	77.2
5	21.2	19.6
6	12.3	10.9
7	109.0	105.0
8	59.3	56.8
9	57.3	56.2
10	47.2	44.2
11	39.3	35.2
12	38.1	37.2
13	27.3	26.8
14	90.2	89.3
15	111.0	106.0

SOLUTION:

Using linear regression method calculate regression parameters:

No. of results – *n*	15	
b	1.021	
a	0.95	ppb
SD_{xy}	1.24	
SD_a	0.70	ppb
SD_b	0.010	
r	0.999	

Check statistically significant differences between parameters ***b*** and 1 and parameters ***a*** and 0 and apply the Student's *t* test.

$$t_b = \frac{|b-1|}{SD_b}$$

$$t_a = \frac{|a|}{SD_a}$$

t_b	2.041
t_a	1.364
t_{crit}	2.160

Because $t_b < t_{crit}$ and $t_a < t_{crit}$, there is no statistically significant difference in the results obtained by both methods.

Conclusion: The results obtained by the investigated method do not differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_6.xls

Example 10.7

Problem: Determine the equivalence of the examined method based on the given series of measurement results for real samples obtained by the examined method and the reference method. Apply the linear regression method.

Data: result series, g/L:

	EXAMINED METHOD	REFERENCE METHOD
	y	*x*
1	12.3	11.6
2	9.4	8.8
3	3.2	2.7
4	15.8	14.9
5	17.4	15.9
6	21.0	19.3
7	21.3	19.6
8	33.8	30.1

SOLUTION:
Using the linear regression method, calculate regression parameters:

No. of results – *n*	8	
b	1.119	
a	–0.42	g/L
SD_{xy}	0.41	
SD_a	0.32	g/L
SD_b	0.019	
r	0.999	

Check statistically significant differences between parameters ***b*** and 1 and parameters ***a*** and 0 and apply the Student's *t* test.

$$t_b = \frac{|b-1|}{SD_b}$$

$$t_a = \frac{|a|}{SD_a}$$

t_b	6.330
t_a	1.299
t_{crit}	2.447

Because $t_b > t_{crit}$ and $t_a < t_{crit}$, there is statistically significant difference in results obtained by both methods.

Conclusion: The results obtained by the investigated method differ statistically significantly from the results obtained by the reference method.

Excel file: exampl_10_7.xls

10.3 Conclusions

Changes and differences in analytical methods may cause significant changes in the obtained results. A comparison of the two methods (and in fact their metrological parameters) can demonstrate their equivalence or lack thereof. In such cases, it is needed to assess the equivalence of the results achieved by the two methods. Equivalence test offers benefits compared to only check validation parameters because the criteria to determine the correctness of a single method does not mean always the identity of the two independent methods, but their compliance [1].

References

1. Chambers D., Kelly G., Limentani G., Lister A., Lung.K.R., and Warner E., Analytical method equivalency an acceptable analytical practice, *Pharm. Techn*., 29(9), 64–80, September 2005.
2. Lung K.R., Gorko M.A., Llewelyn J., and Wiggins N., Statistical method for the determination of equivalence of automated test procedures, *J. Autom. Methods Manag. Chem*., 25(6), 123–127, 2003.
3. Ermer J., Limberger M., Lis K., and Wätzig H., The transfer of analytical procedures, *J. Pharmaceut. Biomed*., 85, 262–276, 2013.
4. Rogers J.L., Howard K.I., and Vessey J.T., Using significance tests to evaluate equivalence between two experimental groups, *Psychol. Bull*., 113, 553–565, 1993.
5. Stegner B.L., Bostrom AG., and Greenfield T.K., Equivalence testing for use in psychosocial and services research: an introduction with examples, *Eval. Program Plann*., 19(3), 193–198, 1996.

Appendix

Table A.1 Critical Values, Student's *t* Test

f \ α	0.05	0.01	*f* \ α	0.05	0.01
1	12.706	63.567	**18**	2.101	2.878
2	4.303	9.925	**19**	2.093	2.861
3	3.182	5.841	**20**	2.086	2.845
4	2.776	4.604	**22**	2.074	2.819
5	2.571	4.032	**24**	2.064	2.797
6	2.447	3.707	**26**	2.056	2.779
7	2.365	3.499	**28**	2.048	2.763
8	2.306	3.355	**30**	2.042	2.750
9	2.262	3.250	**35**	2.030	2.716
10	2.228	3.169	**40**	2.021	2.706
11	2.201	3.106	**45**	2.014	2.690
12	2.179	3.055	**50**	2.009	2.678
13	2.160	3.012	**60**	2.000	2.660
14	2.149	2.977	**70**	1.994	2.648
15	2.131	2.947	**80**	1.990	2.639
16	2.120	2.921	**100**	1.984	2.626
17	2.110	2.898	∞	1.960	2.576

Table A.2 Critical Values of Parameter w_α

f \ α	0.05	0.01
1	1.409	1.414
2	1.645	1.715
3	1.757	1.918
4	1.814	2.051
5	1.848	2.142
6	1.870	2.208
7	1.885	2.256
8	1.895	2.294
9	1.903	2.324
10	1.910	2.348
11	1.916	2.368
12	1.920	2.385
13	1.923	2.399
14	1.926	2.412
15	1.928	2.423
16	1.931	2.432
17	1.933	2.440
18	1.935	2.447
19	1.936	2.454
20	1.937	2.460
22	1.940	2.470
24	1.941	2.479
26	1.943	2.487
28	1.944	2.492
30	1.945	2.498
35	1.948	2.509
40	1.949	2.518
45	1.950	2.524
50	1.951	2.529
60	1.953	2.537
70	1.954	2.542
80	1.955	2.547
100	1.956	2.553
∞	1.960	2.576

Table A.3 Critical Values of z Parameter for Significance Level $\alpha = 0.05$

f \ n	2	3	4	5	6	7	8	9	10	11	12
1	18.0	27.0	32.8	37.1	40.4	43.1	45.4	47.4	49.1	50.6	53.0
5	3.64	4.60	5.22	5.67	6.03	6.33	6.58	6.80	6.99	7.17	7.32
10	3.15	3.88	4.33	4.65	4.91	5.12	5.30	5.46	5.60	5.72	5.83
15	3.01	3.67	4.08	4.37	4.60	4.78	4.94	5.08	5.20	5.31	5.40
20	2.95	3.58	3.96	4.23	4.45	4.62	4.77	4.90	5.01	5.11	5.20
30	2.89	3.49	3.84	4.10	4.30	4.46	4.60	4.72	4.83	4.92	5.00
40	2.86	3.44	3.79	4.04	4.23	4.39	4.52	4.63	4.74	4.82	4.91
60	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65	4.73	4.81
120	2.80	3.36	3.69	3.92	4.10	4.24	4.36	4.48	4.56	4.64	4.72
∞	2.77	3.31	3.63	3.86	4.03	4.17	4.29	4.39	4.47	4.55	4.62

Table A.4 Critical Values of Parameter z_α

n \ α	0.10	0.05	0.01
2	2.06	2.46	3.23
3	1.71	1.96	2.43
4	1.57	1.76	2.14
5	1.50	1.66	1.98

Table A.5 Critical Values (Q_{crit}) of Dixon's Q Test

f \ α	0.10	0.05	0.01
3	0.886	0.941	0.988
4	0.679	0.765	0.889
5	0.557	0.642	0.780
6	0.482	0.560	0.698
7	0.434	0.507	0.637
8	0.399	0.468	0.590
9	0.370	0.437	0.555
10	0.349	0.412	0.527

Table A.6 Critical Values (Q_{crit}) of Dixon's Q Test (Modification for $n \leq 40$)

f \ α	0.05	0.01
3	0.970	0.994
4	0.829	0.926
5	0.710	0.821
6	0.628	0.740
7	0.569	0.680
8	0.608	0.717
9	0.564	0.672
10	0.530	0.635
11	0.502	0.605
12	0.479	0.579
13	0.611	0.697
14	0.586	0.670
15	0.565	0.647
16	0.546	0.627
17	0.529	0.610
18	0.514	0.594
19	0.501	0.580
20	0.489	0.567
21	0.478	0.555
22	0.468	0.544
23	0.459	0.535
24	0.451	0.526
25	0.443	0.517
26	0.436	0.510
27	0.429	0.502
28	0.423	0.495
29	0.417	0.489
30	0.412	0.483
31	0.407	0.477
32	0.402	0.472
33	0.397	0.467
34	0.393	0.462
35	0.388	0.458
36	0.384	0.454
37	0.381	0.450
38	0.377	0.446
39	0.374	0.442
40	0.371	0.438

Table A.7 Critical Values χ^2 Test

f \ α	0.05	0.01
1	3.84	6.64
2	5.99	9.21
3	7.81	11.34
4	9.49	13.28
5	11.07	15.09
6	12.59	16.81
7	14.07	18.48
8	15.51	20.09
9	16.92	21.67
10	18.31	23.21
11	19.68	24.72
12	21.03	26.22
13	22.36	27.69
14	23.68	29.14
15	25.00	30.58
16	26.30	32.00
17	27.59	33.41
18	28.87	34.80
19	30.14	36.19
20	31.41	37.57
21	32.67	38.93
22	33.92	40.29
23	35.17	41.64
24	36.41	42.98
25	37.65	44.31

Table A.8 Critical Values, *Snedecor's F* Test for Significance Level $\alpha = 0.05$ (Top Row) and $\alpha = 0.01$ (Bottom Row)

f_2 \ f_1	2	3	4	5	6	7	8	9	10	11
2	19.00	19.16	19.25	19.30	19.33	19.36	19.37	19.38	19.39	19.40
	99.01	99.17	99.25	99.30	99.33	99.34	99.36	99.38	99.40	99.41
3	9.55	9.28	9.12	9.01	8.94	8.88	8.84	8.81	8.78	8.76
	30.81	29.46	28.71	28.24	27.91	27.67	27.49	27.34	27.23	27.13
4	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.93
	18.00	16.69	15.98	15.52	15.21	14.98	14.80	14.66	14.54	14.45
5	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.78	4.74	4.70
	13.27	12.06	11.39	10.97	10.67	10.45	10.27	10.15	10.05	9.96
6	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03
	10.92	9.78	9.15	8.57	8.47	8.26	8.10	7.98	7.87	7.79
7	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.63	3.60
	9.55	8.45	7.85	7.46	7.19	7.00	6.84	6.71	6.62	6.54
8	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.34	3.31
	8.65	7.59	7.01	6.63	6.37	6.19	6.03	5.91	5.82	5.74
9	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.13	3.10
	8.02	6.99	6.42	6.06	5.80	5.62	5.47	5.35	5.26	5.18
10	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.97	2.94
	7.56	6.55	5.99	5.64	5.39	5.21	5.06	4.95	4.85	4.78
11	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.86	2.82
	7.20	6.22	5.67	5.32	5.07	4.88	4.74	4.63	4.54	4.46

Table A.9 Critical Values, *Hartley's* F_{max} Test for Significance Level $\alpha = 0.05$

f \ k	2	3	4	5	6	7	8	9	10	11
2	39.0	87.5	142	202	266	333	403	475	550	626
3	15.4	27.8	39.2	50.7	62.0	72.9	83.5	93.9	104	114
4	9.60	15.5	20.6	25.2	29.5	33.6	37.5	41.1	44.6	48.0
5	7.15	10.8	13.7	16.3	18.7	20.8	22.9	24.7	26.5	28.2
6	5.82	8.38	10.4	12.1	13.7	15.0	16.3	17.5	18.6	19.7
7	4.99	6.94	8.44	9.70	10.8	11.8	12.7	13.5	14.3	15.1
8	4.43	6.00	7.18	8.12	9.03	9.78	10.5	11.1	11.7	12.2
9	4.03	5.34	6.31	7.11	7.80	8.41	8.95	9.45	9.91	10.3
10	3.72	4.85	5.67	6.34	6.92	7.42	7.87	8.29	8.66	9.01
15	2.86	3.54	4.01	4.37	4.68	4.95	5.19	5.40	5.59	5.77
20	2.46	2.95	3.29	3.54	3.76	3.94	4.10	4.24	4.37	4.49
30	2.07	2.40	2.61	2.78	2.91	3.02	3.12	3.21	3.29	3.36
60	1.67	1.85	1.96	2.04	2.11	2.17	2.22	2.26	2.30	2.33
∞	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table A.10 Critical Values v_o of *Aspin-Welch* Test for Significance Level $\alpha = 0.05$

f_1	f_2 \ c	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
6	6	1.94	1.90	1.85	1.80	1.76	1.74	1.76	1.80	1.85	1.90	1.94
	8	1.94	1.90	1.85	1.80	1.76	1.73	1.74	1.76	1.79	1.82	1.86
	10	1.94	1.90	1.85	1.80	1.76	1.73	1.73	1.74	1.76	1.78	1.81
	15	1.94	1.90	1.85	1.80	1.76	1.73	1.71	1.71	1.72	1.73	1.75
	20	1.94	1.90	1.85	1.80	1.76	1.73	1.71	1.70	1.70	1.71	1.72
	∞	1.94	1.90	1.85	1.80	1.76	1.72	1.69	1.67	1.66	1.65	1.64
8	6	1.86	1.82	1.79	1.76	1.74	1.73	1.76	1.80	1.85	1.90	1.94
	8	1.86	1.82	1.79	1.76	1.73	1.73	1.73	1.76	1.79	1.82	1.86
	10	1.86	1.82	1.79	1.76	1.73	1.72	1.72	1.74	1.76	1.78	1.81
	15	1.86	1.82	1.79	1.76	1.73	1.71	1.71	1.71	1.72	1.73	1.75
	20	1.86	1.82	1.79	1.76	1.73	1.71	1.70	1.70	1.70	1.71	1.72
	∞	1.86	1.82	1.79	1.75	1.72	1.70	1.68	1.66	1.65	1.65	1.64
10	6	1.81	1.78	1.76	1.74	1.73	1.73	1.76	1.80	1.85	1.90	1.94
	8	1.81	1.78	1.76	1.74	1.72	1.72	1.73	1.76	1.79	1.82	1.86
	10	1.81	1.78	1.76	1.73	1.72	1.71	1.72	1.73	1.76	1.78	1.81
	15	1.81	1.78	1.76	1.73	1.72	1.70	1.70	1.71	1.72	1.73	1.75
	20	1.81	1.78	1.76	1.73	1.71	1.70	1.69	1.69	1.70	1.71	1.72
	∞	1.81	1.78	1.76	1.73	1.71	1.69	1.67	1.66	1.65	1.65	1.64
15	6	1.75	1.73	1.72	1.71	1.71	1.73	1.76	1.80	1.85	1.90	1.94
	8	1.75	1.73	1.72	1.71	1.71	1.71	1.73	1.76	1.79	1.82	1.86
	10	1.75	1.73	1.72	1.71	1.71	1.70	1.72	1.73	1.76	1.78	1.81
	15	1.75	1.73	1.72	1.70	1.70	1.69	1.70	1.70	1.72	1.73	1.75
	20	1.75	1.73	1.72	1.70	1.69	1.69	1.69	1.69	1.70	1.71	1.72
	∞	1.75	1.73	1.72	1.70	1.68	1.67	1.66	1.65	1.65	1.65	1.64
20	6	1.72	1.71	1.70	1.70	1.71	1.73	1.76	1.80	1.85	1.90	1.94
	8	1.72	1.71	1.70	1.70	1.70	1.71	1.73	1.76	1.79	1.82	1.86
	10	1.72	1.71	1.70	1.69	1.69	1.70	1.71	1.73	1.76	1.78	1.81
	15	1.72	1.71	1.70	1.69	1.69	1.69	1.69	1.70	1.72	1.73	1.75
	20	1.72	1.71	1.70	1.69	1.68	1.68	1.68	1.69	1.70	1.71	1.72
	∞	1.72	1.71	1.70	1.68	1.67	1.66	1.66	1.65	1.65	1.65	1.64
∞	6	1.64	1.65	1.66	1.67	1.69	1.72	1.76	1.80	1.85	1.90	1.94
	8	1.64	1.65	1.65	1.66	1.68	1.70	1.72	1.75	1.79	1.82	1.86
	10	1.64	1.65	1.65	1.66	1.67	1.69	1.71	1.73	1.76	1.78	1.81
	15	1.64	1.65	1.65	1.65	1.66	1.67	1.68	1.70	1.72	1.73	1.75
	20	1.64	1.65	1.65	1.65	1.66	1.66	1.67	1.68	1.70	1.71	1.72
	∞	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64

Table A.11 Critical Values of *Cochran's* Test

p	$n = 2$		$n = 3$		$n = 4$		$n = 5$		$n = 6$	
	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.05$
2	–	–	0.995	0.975	0.979	0.939	0.959	0.906	0.937	0.877
3	0.993	0.967	0.942	0.871	0.883	0.798	0.834	0.746	0.793	0.707
4	0.968	0.906	0.864	0.768	0.781	0.684	0.721	0.629	0.676	0.590
5	0.928	0.841	0.788	0.684	0.696	0.598	0.633	0.544	0.588	0.506
6	0.883	0.781	0.722	0.616	0.626	0.532	0.564	0.480	0.520	0.445
7	0.838	0.727	0.664	0.561	0.568	0.480	0.508	0.431	0.466	0.397
8	0.794	0.680	0.615	0.516	0.521	0.438	0.463	0.391	0.423	0.360
9	0.754	0.638	0.573	0.478	0.481	0.403	0.425	0.358	0.387	0.329
10	0.718	0.602	0.536	0.445	0.447	0.373	0.393	0.331	0.357	0.303
11	0.684	0.570	0.504	0.417	0.418	0.348	0.366	0.308	0.332	0.281
12	0.653	0.541	0.475	0.392	0.392	0.326	0.343	0.288	0.310	0.262
13	0.624	0.515	0.450	0.371	0.369	0.307	0.322	0.271	0.291	0.243
14	0.599	0.492	0.427	0.352	0.349	0.291	0.304	0.255	0.274	0.232
15	0.575	0.471	0.407	0.335	0.332	0.276	0.288	0.242	0.259	0.220
16	0.553	0.452	0.388	0.319	0.316	0.262	0.274	0.230	0.246	0.208
17	0.532	0.434	0.372	0.305	0.301	0.250	0.261	0.219	0.234	0.198
18	0.514	0.418	0.356	0.293	0.288	0.240	0.249	0.209	0.223	0.189
19	0.496	0.403	0.343	0.281	0.276	0.230	0.238	0.200	0.214	0.181
20	0.480	0.389	0.330	0.270	0.265	0.220	0.229	0.192	0.205	0.174
21	0.465	0.377	0.318	0.261	0.255	0.212	0.220	0.185	0.197	0.167
22	0.450	0.365	0.307	0.252	0.246	0.204	0.212	0.178	0.189	0.160
23	0.437	0.354	0.297	0.243	0.238	0.197	0.204	0.172	0.182	0.155
24	0.425	0.343	0.287	0.235	0.230	0.191	0.197	0.166	0.176	0.149
25	0.413	0.334	0.278	0.228	0.222	0.185	0.190	0.160	0.170	0.144
26	0.402	0.325	0.270	0.221	0.215	0.179	0.184	0.155	0.164	0.140
27	0.391	0.316	0.262	0.215	0.209	0.173	0.179	0.150	0.159	0.135
28	0.382	0.308	0.255	0.209	0.202	0.168	0.173	0.146	0.154	0.131
29	0.372	0.300	0.248	0.203	0.196	0.164	0.168	0.142	0.150	0.127
30	0.363	0.293	0.241	0.198	0.191	0.159	0.164	0.138	0.145	0.124
31	0.355	0.286	0.235	0.193	0.186	0.155	0.159	0.134	0.141	0.120
32	0.347	0.280	0.229	0.188	0.181	0.151	0.155	0.131	0.138	0.117
33	0.339	0.273	0.224	0.184	0.177	0.147	0.151	0.127	0.134	0.114
34	0.332	0.267	0.218	0.179	0.172	0.144	0.147	0.124	0.131	0.111
35	0.325	0.262	0.213	0.175	0.168	0.140	0.144	0.121	0.127	0.108
36	0.318	0.256	0.208	0.172	0.165	0.137	0.140	0.118	0.124	0.106
37	0.312	0.251	0.204	0.168	0.161	0.134	0.137	0.116	0.121	0.103
38	0.306	0.246	0.200	0.164	0.157	0.131	0.134	0.113	0.119	0.101
39	0.300	0.242	0.196	0.161	0.154	0.129	0.131	0.111	0.116	0.099
40	0.294	0.237	0.192	0.158	0.151	0.126	0.128	0.108	0.114	0.097

p – number of laboratories

n – number of results for one level

Table A.12 Critical Values of *Grubbs'* Test

p	ONE GREATEST AND ONE SMALLEST		TWO GREATEST AND TWO SMALLEST	
	UPPER $\alpha = 0.01$	LOWER $\alpha = 0.05$	UPPER $\alpha = 0.01$	LOWER $\alpha = 0.05$
3	1.155	1.155	–	–
4	1.496	1.481	0.000 0	0.000 2
5	1.764	1.715	0.001 8	0.009 0
6	1.973	1.887	0.011 6	0.034 9
7	2.139	2.020	0.030 8	0.070 8
8	2.274	2.126	0.056 3	0.110 1
9	2.387	2.215	0.085 1	0.149 2
10	2.482	2.290	0.115 0	0.186 4
11	2.564	2.335	0.144 8	0.221 3
12	2.636	2.412	0.173 8	0.253 7
13	2.699	2.462	0.201 6	0.283 6
14	2.755	2.507	0.228 0	0.311 2
15	2.806	2.549	0.253 0	0.336 7
16	2.852	2.585	0.276 7	0.360 3
17	2.894	2.620	0.299 0	0.382 2
18	2.932	2.651	0.320 0	0.402 5
19	2.968	2.681	0.339 8	0.421 4
20	3.001	2.709	0.358 5	0.439 1
21	3.031	2.733	0.376 1	0.455 6
22	3.060	2.758	0.392 7	0.471 1
23	3.087	2.781	0.408 5	0.485 7
24	3.112	2.802	0.423 4	0.499 4
25	3.135	2.822	0.437 6	0.512 3
26	3.157	2.841	0.451 0	0.524 5
27	3.178	2.859	0.463 8	0.536 0
28	3.199	2.876	0.475 9	0.547 0
29	3.218	2.893	0.487 5	0.557 4
30	3.236	2.908	0.498 5	0.567 2
31	3.253	2.924	0.509 1	0.576 6
32	3.270	2.938	0.519 2	0.585 6
33	3.286	2.952	0.528 8	0.594 1
34	3.301	2.965	0.538 1	0.602 3
35	3.316	2.979	0.546 9	0.610 1
36	3.330	2.991	0.555 4	0.617 5
37	3.343	3.003	0.563 6	0.624 7
38	3.356	3.014	0.571 4	0.631 6
39	3.369	3.025	0.578 9	0.638 2
40	3.381	3.036	0.586 2	0.644 5

p – number of laboratories

Table A.13A Parameters *h* and *k Mandel's* Test for Significance Level $\alpha = 0.01$

p	*h*	*k*								
		n								
		2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	1.15	1.71	1.64	1.58	1.53	1.49	1.46	1.43	1.41	1.39
4	1.49	1.91	1.77	1.67	1.60	1.55	1.51	1.48	1.45	1.43
5	1.72	2.05	1.85	1.73	1.65	1.59	1.55	1.51	1.48	1.46
6	1.87	2.14	1.90	1.77	1.68	1.62	1.57	1.53	1.50	1.47
7	1.98	2.20	1.94	1.79	1.70	1.63	1.58	1.54	1.51	1.48
8	2.06	2.25	1.97	1.81	1.71	1.65	1.59	1.55	1.52	1.49
9	2.13	2.29	1.99	1.82	1.73	1.66	1.60	1.56	1.53	1.50
10	2.18	2.32	2.00	1.84	1.74	1.66	1.61	1.57	1.53	1.50
11	2.22	2.34	2.01	1.85	1.74	1.67	1.62	1.57	1.54	1.51
12	2.25	2.36	2.02	1.85	1.75	1.68	1.62	1.58	1.54	1.51
13	2.27	2.38	2.03	1.86	1.76	1.68	1.63	1.58	1.55	1.52
14	2.30	2.39	2.04	1.87	1.76	1.69	1.63	1.58	1.55	1.52
15	2.32	2.41	2.05	1.87	1.76	1.69	1.63	1.59	1.55	1.52
16	2.33	2.42	2.05	1.88	1.77	1.69	1.63	1.59	1.55	1.52
17	2.35	2.44	2.06	1.88	1.77	1.69	1.64	1.59	1.55	1.52
18	2.36	2.44	2.06	1.88	1.77	1.70	1.64	1.59	1.56	1.52
19	2.37	2.44	2.07	1.89	1.78	1.70	1.64	1.59	1.56	1.53
20	2.39	2.45	2.07	1.89	1.78	1.70	1.64	1.60	1.56	1.53
21	2.39	2.46	2.07	1.89	1.78	1.70	1.64	1.60	1.56	1.53
22	2.40	2.46	2.08	1.90	1.78	1.70	1.65	1.60	1.56	1.53
23	2.41	2.47	2.08	1.90	1.78	1.71	1.65	1.60	1.56	1.53
24	2.42	2.47	2.08	1.90	1.79	1.71	1.65	1.60	1.56	1.53
25	2.42	2.47	2.08	1.90	1.79	1.71	1.65	1.60	1.56	1.53
26	2.43	2.48	2.09	1.90	1.79	1.71	1.65	1.60	1.56	1.53
27	2.44	2.48	2.09	1.90	1.79	1.71	1.65	1.60	1.56	1.53
28	2.44	2.49	2.09	1.91	1.79	1.71	1.65	1.60	1.57	1.53
29	2.45	2.49	2.09	1.91	1.79	1.71	1.65	1.60	1.57	1.53
30	2.45	2.49	2.10	1.91	1.79	1.71	1.65	1.61	1.57	1.53

p – number of laboratories

n – number of results for one level

Table A.13B Parameters *h* and *k Mandel'* Test for Significance Level $\alpha = 0.05$

p	*h*	*k*								
		n								
		2	3	4	5	6	7	8	9	10
3	1.15	1.65	1.53	1.45	1.40	1.37	1.34	1.32	1.30	1.29
4	1.42	1.76	1.59	1.50	1.44	1.40	1.37	1.35	1.33	1.31
5	1.57	1.81	1.62	1.53	1.46	1.42	1.39	1.36	1.34	1.32
6	1.66	1.85	1.64	1.54	1.48	1.43	1.40	1.37	1.35	1.33
7	1.71	1.87	1.66	1.55	1.49	1.44	1.41	1.38	1.36	1.34
8	1.75	1.88	1.67	1.56	1.50	1.45	1.41	1.38	1.36	1.34
9	1.78	1.90	1.68	1.57	1.50	1.45	1.42	1.39	1.36	1.35
10	1.80	1.90	1.68	1.57	1.50	1.46	1.42	1.39	1.37	1.35
11	1.82	1.91	1.69	1.58	1.51	1.46	1.42	1.39	1.37	1.35
12	1.83	1.92	1.69	1.58	1.51	1.46	1.42	1.40	1.37	1.35
13	1.84	1.92	1.69	1.58	1.51	1.46	1.43	1.40	1.37	1.35
14	1.85	1.92	1.70	1.59	1.52	1.47	1.43	1.40	1.37	1.35
15	1.86	1.93	1.70	1.59	1.52	1.47	1.43	1.40	1.38	1.36
16	1.86	1.93	1.70	1.59	1.52	1.47	1.43	1.40	1.38	1.36
17	1.87	1.93	1.70	1.59	1.52	1.47	1.43	1.40	1.38	1.36
18	1.88	1.93	1.71	1.59	1.52	1.47	1.43	1.40	1.38	1.36
19	1.88	1.93	1.71	1.59	1.52	1.47	1.43	1.40	1.38	1.36
20	1.89	1.94	1.71	1.59	1.52	1.47	1.43	1.40	1.38	1.36
21	1.89	1.94	1.71	1.60	1.52	1.47	1.44	1.41	1.38	1.36
22	1.89	1.94	1.71	1.60	1.52	1.47	1.44	1.41	1.38	1.36
23	1.90	1.94	1.71	1.60	1.53	1.47	1.44	1.41	1.38	1.36
24	1.90	1.94	1.71	1.60	1.53	1.48	1.44	1.41	1.38	1.36
25	1.90	1.94	1.71	1.60	1.53	1.48	1.44	1.41	1.38	1.36
26	1.90	1.94	1.71	1.60	1.53	1.48	1.44	1.41	1.38	1.36
27	1.91	1.94	1.71	1.60	1.53	1.48	1.44	1.41	1.38	1.36
28	1.91	1.94	1.71	1.60	1.53	1.48	1.44	1.41	1.38	1.36
29	1.91	1.94	1.72	1.60	1.53	1.48	1.44	1.41	1.38	1.36
30	1.91	1.94	1.72	1.60	1.53	1.48	1.44	1.41	1.38	1.36

p – number of laboratories

n – number of results for one level

Table A.14 Critical Values (λ_α) *Kolmogorov-Smirnov* Test

α	λ_α
0.01	1.63
0.02	1.52
0.05	1.36
0.10	1.22
0.15	1.14
0.20	1.07
0.25	1.02
0.30	0.97
0.40	0.89
0.50	0.83
0.60	0.77
0.70	0.71
0.80	0.64
0.90	0.57
0.99	0.44

Table A.15 Critical Values of Regression Coefficient r_{crit}

α / *f*	**0.05**	**0.01**
5	0.75	0.87
6	0.71	0.83
7	0.67	0.80
8	0.63	0.77
9	0.60	0.74
10	0.58	0.71
12	0.53	0.66
14	0.50	0.62
16	0.47	0.59
18	0.44	0.56
20	0.42	0.54
25	0.38	0.49
30	0.35	0.45
40	0.30	0.39
50	0.27	0.35
60	0.25	0.33
80	0.22	0.28
100	0.20	0.25

Index

T

U

V

Y

Z